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CURRENT CANCER RESEARCH

on

INTERFERON AND OTHER ANTICANCER AND ANTIVIRAL AGENTS

FEBRUARY 1981

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
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This **SPECIAL LISTING** of Current Cancer Research Projects is a publication of the INTERNATIONAL CANCER RESEARCH DATA BANK (ICRDB) Program of the National Cancer Institute. Each **LISTING** contains descriptions of ongoing projects in one selected cancer research area. Approximately 50 different cancer research areas are covered by a separate **SPECIAL LISTING**.

The present series of **SPECIAL LISTINGS** is a third edition, and begins with number 201. This series will update the second edition which included **SPECIAL LISTINGS** numbered 101 through 147. Depending on the total number and types of cancer research summaries received, the third edition will have at least 47 titles, and possibly a few more. In most cases, each third edition **SPECIAL LISTING** will replace the exact subject area of a second edition **LISTING** (SL 201 replacing SL 101, etc.). Whenever the number and variety of summaries requires a change in this procedure, a notation will be made in the specific **LISTINGS** affected.

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I. INTERFERON

A. INTERFERON THERAPY OF MALIGNANT DISEASES

1. MEASUREMENT OF INTERFERON LEVELS AND SENSITIVITY OF LEUKEMIC BLASTS TO INTERFERON IN AML PATIENTS GIVEN INTERFERON I.V. (ABBREV)

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A phase 1 toxicity trial to test human lymphoblastoid (Nama 1 wa) interferon in the treatment of A.M.L. patients has recently started at the ICRF Medical Oncology Unit, St. Bartholomew's Hospital. In parallel to this, a collaborative project between laboratories at Lincoln's Inn Fields and laboratories at St. Bartholomew's Hospital has begun to investigate the pharmacokinetics and effects of this interferon in vivo and in vitro. A sensitive and fast bioassay for serum interferon has been developed which measures the inhibition of viral RNA synthesis 6 hours after injection. Using this method, we have established that the first trial patient attained high levels of serum interferon 30 minutes to two hours after intravenous injection. As further patients enter the trial, more pharmacokinetic studies will be possible.

A microculture system for the short term culture of leukemia cells has been developed (Balkwill and Oliver, International Journal of Cancer, 1977, 20, 500) and this assay is being used to determine the in vitro sensitivity to interferon of the pre-treatment bone marrow and peripheral blood leukemia cells of each trial patient. A study using the first patient has shown that inhibition of cell growth in vitro could be obtained with doses of interferon that were attained in vivo after intravenous injection.

In trial patients with relatively normal levels of peripheral blood lymphocytes, investigations into natural killer cell activity in pre-and post-interferon treatment blood will be carried out to see whether these doses of interferon can stimulate the host immune system.

2. STUDIES ON THE TREATMENT OF DISEASE WITH THE INTERFERON SYSTEM

Levy, H. B., Riley, F., Levine, A., Engel, K., Hooks, J., Stephen, E., Liu, C., Harrington, D., Lerner, M., Crane, L., Oettgen, H., Krown, S., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Inst. of Allergy & Infectious Diseases, Molecular Virology Section, *9000 Rockville Pike, Bethesda, Maryland,* 20205, United States of America

The nuclease-resistant, primate-effective interferon inducer, poly inosinic polycytidylic acid, poly lysine, carboxymethyl cellulose (Poly ICLC), has a variety of physiological activities. In addition to inducing interferon in primates, it is an immune adjuvant and a radioprotective agent. It is currently being evaluated in 3 collaborative clinical studies, and 3 more are being developed. It appears to have had ameliorative effects in 4 patients; one with lymphoblastic leukemia, one cancer patient whose widely disseminated herpes lesions disappeared after treatment, and 2 patients with chronic relapsing polyneuropathy. A drug without carboxymethylcellulose has been prepared and is being evaluated.

BIBLIOGRAPHIC REFERENCES: Harrington, D. G., Crabbs, C. L., Hilmas, D. E., Brown, R., Jr., Higbee, S. A., Cole, E., Levy, H.B.: Adjuvant effects of low doses of a nuclease-resistant derivative of polyinosinic acid on antibody responses of monkeys to inactivated Venezuelan equine encephalomyelitis virus vaccine. Infection and Immunity, Apr. 1979, p. 160-166. Levy, H. B., Hilmas, D. E. Evaluation of a nuclease-resistant derivative of PICLC as a radioprotective agent. Radiation Research, 77, 1979.

3. INTERFERON TREATMENT IN LEUKEMIA

Greenberg, P. L., Merigan, T. C., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, Medical Service, *3801 Junipero Serra Blvd., Palo Alto, California,* 94304, United States of America

Interferon inhibits normal and neoplastic cells proliferating in experimental animals and in preliminary clinical trials in man. Alternative forms of treatment are needed for patients with chronic myeloid leukemia (CML) since no essential change in the natural history of the disease has occurred in the past 50 years. The median survival of these patients is approximately 3.5 years due to the 80% incidence of transformation into a refractory phase known as blast crisis. Concern has been raised as to whether potentially oncogenic drugs such as Busulfan may contribute to the high incidence of transformation. No such association has been found with interferon. With this in view we plan to evaluate the effects of interferon on leukemic cell mass in vivo and in vitro.

To date no patient with CML has been studied due to lack of availability of interferon. We have recently joined Dr. T. Merigan in submitting to the NCI a proposal to obtain interferon for a Phase I/II trial evaluating the drug in patients with CML.

4. HUMAN LEUKOCYTE TREATMENT OF PATIENTS WITH VARIOUS MALIGNANT DISEASES

Strander, H., Nilsonne, U., Karolinska Sjukhuset, *S10401 Stockholm, Sweden*

Continuation of the clinical trial of interferon treatment for osteosarcoma: Patients admitted to the Karolinska Hospital since 1971 are all receiving interferon. Prognostic factors, metastases and survival compared to a concurrent control group consisting of all the other cases of osteosarcoma treated in Sweden during the same time. At present the frequency of metastases is about 50 percent in the interferon treated group as compared to the control group. A similar difference is obtained as regards survival.

A pilot study is done on juvenile laryngeal papilloma where patients exhibiting progressing tumor growth are treated with interferon. Regression has been demonstrated in 7 so treated cases. Upon removal of interferon progression again occurred. Regression has again been demonstrated after resumption of interferon administration.

Myeloma pilot study: Interferon has been shown to cause regression of multiple myeloma, even complete remissions. A pilot study is now continuing including also melphalan resistant myeloma cases.

A randomized study on multiple myeloma has been initiated where interferon therapy is compared to conventional melphalan-prednisolone treatment.

Studies of the effect of interferon therapy on malignant melanoma and ovarian cancer are to be initiated.

5. INTERFERON TREATMENT OF A MURINE OSTEOGENIC SARCOMA

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Interferon will be evaluated for its antineoplastic activity in the prevention and treatment of murine osteogenic sarcoma. The efficacy of interferon will be determined: (1) In tissue culture against a continuous line of osteogenic sarcoma cells derived from a 239 Pu-induced osteogenic sarcoma in C57BL/6 mice, (2) in an in vivo model utilizing osteogenic sarcoma tumor cells implanted subcutaneously into syngeneic mice, and (3) in lung metastases established by intravenous inoculation of the continuous line of osteogenic sarcoma cells. Based on the efficacy of these initial experiments, the capacity of interferon to prevent the induction of osteogenic sarcomas in mice exposed to 239 Pu will now be delineated and the treatment of established tumors will be attempted.

6. INTERFERON TREATMENT OF SPONTANEOUS LYMPHOSARCOMA IN CATS

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This is part of a broader project. A summary of this subproject is not available.

7. INTERFERON THERAPY OF MYELOMATOSIS AND OTHER TUMOR DISEASES

Strander, H., Radiumhemmet, *S10401 Stockholm, Sweden*

OBJECTIVES: A) In a prospective randomized study, comparison is made between intermittent melphalan-prednisolone treatment and interferon therapy against myelomatosis. B) In a pilot study different doses are compared in the treatment of juvenile laryngeal papillomatosis with interferon therapy. C) Pilot studies with interferon therapy are being done on other tumor diseases.

APPROACH: A) Myelomatosis - the medical departments of Huddinge Hospital, Sodersjukhuset, Serafimerlasarettet, Akademiska sjukhuset in Uppsala, Regionsjukhuset in Orebro and Radiumhemmet at the Karolinska Hospital are taking part in a prospective randomized investigation where intermittent melphalan-prednisolone treatment is compared with interferon therapy. If the inteferon therapy arm compares unfavorably with the arm treated with chemotherapy, discussions will take place in order to try increased interferon dosaging and combination therapy with melphalan and interferon. Earlier it was shown at Radiumhemmet that interferon therapy can have an effect on myelomatosis. B) Juvenile laryngeal papillomatosis - a pilot study is being done in cooperation with the ENT section at the Karolinska Hospital. Seven patients treated with interferon have all shown regression. After cessation of interferon therapy a new progression of the disease is revealed. Therefore, a pilot study is being made in order to determine how much interferon is required for these patients in order to prevent recurrence. C) Other tumor diseases - interferon is also being given to patients suffering from oat-cell cancer, ovarian cancer, melanoma and squamous cell carcinoma. D) Pilot studies are also being made in order to see if interferon effects can be potentiated by other biological modifiers.

All these patients are involved in various research programs taking place at different institutions.

PROGRESS: Interferon has an anti-tumor effect on humans. The studies now being conducted aim at studying how one should give the interferon therapy in an optimal way.

8. NATURAL KILLER CELL ACTIVITY DURING INTERFERON THERAPY OF MULTIPLE MYELOMA

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Immunotherapy of cancer with BCG and interferon has been moderately successful in rodents and humans. The mechanism by which these agents cause tumor regression is unknown, but recent findings from our laboratory and others have suggested that both these agents activate natural killer (NK) cells either directly (interferon) or through macrophage intervention (BCG). NK cells spontaneously lyse a wide variety of tumor cells in vitro. We have shown that mouse NK cells from BCG-stimulated mice are very effective in the suppression of a variety of tumors in vivo. Our main goals, therefore, are to elucidate the mechanism by which BCG and interferon activate NK cells and to relate the activation of NK cells to the regression of neoplasms in rodents and humans during immunotherapy.

The studies proposed involve three interrelated projects. First, we will analyze the cellular and molecular components involved in the activation of rodent NK cells by BCG. We will focus on the role of macrophages, interferon, and prostaglandins in this process. Second, we will extend our recent observations that tumor-bearing mouse NK cells are anergic to stimulation by BCG and that this anergy can be overcome with indomethacin, an inhibitor of prostaglandin synthesis. The regulation of NK activity by soluble factors derived from tumor cells and tumor-bearer leukocytes will be evaluated. Also, the pharmacologic basis for the indomethacin effects will be studied. Third, we will measure blood NK activity levels in human multiple myeloma patients involved in a national interferon trial. We will compare the changes in NK activity with the clinical progress of the patients during interferon therapy. In addition, NK cells from these patients will be stimulated in vitro with interferon and indomethacin to obtain information which may lead to improved therapy regimens.

9. HUMAN LEUKOCYTE INTERFERON - MASS PRODUCTION FOR SYSTEMIC ADMINISTRATION IN CANCER PATIENTS

de la Pena, N. C., Bal, E. D., Puricelli, L., Inst. de Oncologia Angel H. Roffo, Dept. de Investigacion, *5481 San Martin Ave., 1417 Buenos Aires, Argentina*

OBJECTIVE: Large-scale production of human leukocyte interferon (HLI). Studies of purity and safety. Standardization methods for the control of the antimitotic and other biological properties of HLI. Clinical use of HLI in cancer patients.

PROGRESS: A) Basic Research Plan: Antiviral and antimitotic effects of HLI. Biochemical approach. Cell morphological changes induced by HLI in tissue culture cell lines. Ultramicroscopic and scanning electron-microscopic evaluations. B) Clinical Research Plan: a) Evaluation of the HLI therapy in cancer patients with herpes zoster infection. Topical versus topical plus systemic therapies are being compared. b) Different protocols for the HLI systemic administration in cancer patients are being prepared.

10. EVALUATION OF THERAPEUTIC EFFICACY OF HUMAN INTERFERON

Merigan, T. C., Stanford University, School of Medicine, Dept. of Medicine, *Stanford, California*, 94305, United States of America

OBJECTIVES: To continue to evaluate the efficacy of partially purified human leukocyte interferon (prepared by Dr. Cantell in Finland and processed, tested, and packaged by Smith, Kline, and French) in patients with disseminated varicella zoster infections. These studies will be done in patients who are immunosuppressed or who have congenital immune deficiencies. Evaluate whether interferon can be effective in the clearance of hepatitits antigen in individuals who are chronic carriers. This study will proceed in a stepwise fashion, testing one patient at a time and evaluating the interferon effect on hepatitis polymerase activity, antigen level, and antibody titer.

11. INTERFERON FOR CMV INFECTION, LEUKEMIC RELAPSE AND GVHD

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Among the major problems influencing the outcome of human marrow transplantation are disseminated cytomegalovirus(CMV) infection, leukemic relapse, and graft-versus-host disease(GVHD). Sixty of the 377 marrow graft recipients developed CMV pneumonia with an 88% mortality. Incidence was 22% among those with leukemia in relapse at the time of transplant.

Graft-versus-host disease occurs in up to 70% of allogeneic graft recipients, and relapse of leukemia occurs in 50% of patients with leukemia. Overall, the one year survival is 30%.

Human leukocyte interferon has properties that could be beneficial for treatment of all 3 conditions. It is antiviral, has been shown to combat GVHD in animal models, and has shown promising preliminary results against neoplastic disease in both animals and man.

This proposal outlines a prospective randomized trial of human leukocyte interferon following allogeneic marrow trans-

plant for leukemia in relapse, in an attempt to prevent or relieve these important complications of marrow transplantation.

12. A NEW INTERFERON SYSTEM ASSAY IN PATIENTS WITH MALIGNANCIES AND SUSPECTED MALIGNANCIES

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The object of this study is to evaluate various parameters of the interferon system, both extracellular and intracellular, in patients with malignancies and those suspected of having malignancy. A combined assay which studies the interferon levels in the blood of the patient and the ability of the patient's polymorphonuclear monocytes (PBMC) to produce both type I and type II interferon when appropriately stimulated and evaluates the anti-viral status of the patient's PBMC, will be performed in all cases. Fifty to one hundred children and adults with neoplastic disease or suspected disease will be studied and correlations will be made between the assay and the clinical status and course of the patients. Preliminary studies indicate that this assay system may be useful in indicating which patients are likely to respond to interferon therapy. These studies in patients receiving interferon therapy have shown the practicability of this research. The immuno-regulatory effect of interferon used in the treatment of the patients is also being studied.

13. PROGRAM PROJECT ON ATHYMIC MICE AND HUMAN TUMORS

Kaplan, N. O., Sato, G. H., Masui, H., Rizzino, A. A., Buonassisi, V., Howell, S. B., University of California, San Diego Campus, School of Medicine, Dept. of Chemistry, *P.O. Box 109, La Jolla, California*, 92093, United States of America

An athymic mouse research facility has been established at the University of California, San Diego. The mouse colony contains close to 5,000 animals. Approximately one half of them are used in experiments with transplantable human tumors. Twenty-seven human tumor lines are being maintained in the mice and in tissue cultures. This number is constantly increasing.

Hormonal requirements of human tumors for growth in serum-free culture are under study. The absence of serum in culture enables one to discover hitherto unsuspected hormonal dependencies of these tumors.

Clinical chemotherapy results in humans are correlated with those in mice or cell cultures. In these studies new and modified therapeutic agents (e.g., interferon and polymeric drugs) potentially useful in humans are being used.

The effects of nutrition and growth requirements on human tumors are also being explored.

B. STUDIES ON ANTICANCER ACTIVITY OF INTERFERON

14. GROWTH AND DIFFERENTIATION OF TUMOR CELLS IN THE PRESENCE OF PHYSIOLOGIC MEDIATORS - STUDIES ON MEMBRANE MARKERS

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OBJECTIVE: Study of the role played by the plasma membrane in controlling the expression of typical differentiation genes in tumor cells. In particular, the effects of interferon, seemingly acting via an interaction with the plasma membrane, will be investigated. In addition, the role of prostaglandins and/or cAMP in the differentiation of murine and human erythroid precursors will be analyzed.

APPROACH: In the past we have studied the effects of interferon on the DMSO-induced erythroid differentiation of Friend leukemia cells; presently we aim at understanding whether the mechanisms of these effects have anything to do with interferon interactions with the plasma membrane, i.e., by studying the effects of cell treatment with purified cholera toxin and/or its B subunit. The effects of prostaglandins will also be studied using the clonal short-term assays termed CFU-E, BFU-E and CFU-C, both in humans and in the mouse.

15. EFFECT OF HUMAN LYMPHOBLASTOID INTERFERON ON THE GROWTH OF HUMAN BREAST CARCINOMAS IN THE NUDE MOUSE

Balkwill, F., Taylor Papadimitriou, J., Sebesteny, A., Fantes, K. H., Raymond, R. H., Good, E., Imperial Cancer Research Fund, Epithelial Cell Growth Regulation Laboratory, *Lincolns Inn Fields, London WC2A 3PX, England, United Kingdom*

Human lymphoblastoid interferon inhibited the growth of two out of three primary human breast carcinomas grown as xenografts in the nude mouse. This interferon, given daily at doses equivalent to those attainable in man, reduced the number of tumors by 84 percent and 63 percent, respectively, in two different xenografts. The inhibition could be abrogated by preincubation of the interferon with a specific antiserum. Interferon may act as an anti-cancer agent: 1. by inhibiting viral oncogenesis; 2. by enhancing host reaction against tumor; or 3. through a direct cytostatic effect on the tumor cells.

We are now using the nude mouse xenograft model system to investigate the mechanism of interferon action. Preliminary evidence from two sources indicates that interferon acts by direct cytostatic effect on the tumor cells. Firstly, human interferon did not stimulate the mouse immune system although mouse interferon significantly enhanced nude mouse natural killer cell activity; and secondly, mouse interferon did not inhibit the growth of human tumors in the mouse.

Further experiments are in progress to elucidate the mechanism of interferon inhibition and to investigate the sensitivity of a range of other tumors.

16. INVESTIGATIONS INTO INTERSPECIFIC COMMUNICATION OF THE INTERFERON INDUCED ANTIVIRAL STATE AND GROWTH INHIBITORY EFFECTS

Balkwill, F., Taylor Papadimitriou, J., Spurr, N., Ito, Y., Imperial Cancer Research Fund, Epithelial Cell Growth Regulation Laboratory, *Lincolns Inn Fields, London WC2A 3PX, England, United Kingdom*

Recent work has suggested that the interferon-induced antiviral state can be transferred interspecifically in mixed cultures of murine and human cells treated with either murine or human interferon (Blalock and Baron, Nature, 1977, 269, 422). In view of the interest of this laboratory in communication between cells by small molecular weight compounds, and the current interest in small molecular weight interferon-induced inhibitors, we set out to investigate this phenomenon further and to see if the cell growth inhibitory effects of interferon could also be transferred from cell to cell. We chose to work with polyoma virus which only grew in the mouse cells, and thus the test system comprised human/mouse mixed cell cultures treated with human interferon and infected with polyoma virus. In this system, we were unable to demonstrate any interspecific communication of the antiviral state, whether T or V antigen synthesis or virus production was measured.

To study communication of the growth inhibitory effects of interferon, we used quiescent cells stimulated to enter one cell cycle. Interferon added at the time of stimulation delayed the entry of cells into mitosis by 6 to 8 hours. (Balkwill and Taylor-Papadimitriou, Nature 1978, 274, 798). In mixed quiescent cultures of human and mouse cells stimulated to divide and treated with one species of interferon, cells from the other species entered mitosis at the same time as control cells, whereas cells from the same species as the interferon were delayed for 6 to 8 hours. Junctional communication between the two cell types was demonstrated by transfer of 3H uridine-labelled nucleotide.

We therefore conclude that in the systems used in this laboratory, the establishment of cell growth inhibition and the antiviral state by interferon does not involve communication of stable small molecular weight mediators between cells.

17. INTERFERON AND GROWTH IN MAMMALIAN CELLS

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Interferons are cellular proteins which inhibit DNA synthesis and division in normal and transformed animal cells. We propose that interferons can be used as experimental tools to

study the control and regulatory processes involved in the proliferation of eukaryotic cells. Interferons display a selective inhibition of the synthesis of certain polypeptides in cells transiting from G1 to S phase. The nature of the above inhibition and the polypeptides involved will be examined to determine their role in initiation of cellular DNA synthesis. The bases for the inhibitory effect of interferons on ornithine decarboxylase, a key enzyme appearing in animal cells during their transit from normal to neoplastic growth, will be studied in detail. The control and regulation of polyamines and the involved biosynthetic enzymes will be investigated using intact cells as well as an 'in vitso' translational system for ornithine decarboxylase.

18. MODULATION OF THE ANTITUMOR EFFECT OF INTERFERON

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Interferon has been shown to have a profound effect on tumor growth, viral replication, and the immune responses. We have identified two new lymphokine activities in mitogen-stimulated mouse spleen cell supernatant fluids which modulate the antiviral activity of interferon. One is a potentiator which enhances the antiviral activity of interferon. A second is an inhibitor which blocks both the antiviral and the immunoregulatory activity of interferon. The crude preparations also contain immune interferon. The potentiator, and immune interferon are not produced by unstimulated mouse spleen cells. We have developed large scale methods of production of potentiator, inhibitor, and immune interferon. In addition, we have purified the inhibitor away from potentiator and immune interferon by sequential chromatography on BSA-Affi-Gel and Ultrogel columns. Such inhibitor preparations contain 0.019 mg protein/ml, have been purified about 1000-fold, and can negate the antiviral activity of at least 400 units of interferon. The inhibitor prevents the establishment of the interferon induced antiviral state when added as late as 3 hrs after interferon, but it does not reverse an established antiviral state. The potentiator has been purified about 200-fold but has not yet been separated from immune interferon. The potentiator enhances the antiviral activity of added fibroblast interferon by a factor of 10. The potentiation factor is either immune interferon itself or a factor which comigrates with immune interferon through a gel filtration column. This study will employ inhibitor and potentiator produced and purified in bulk quantities so they may be characterized and employed to study their modulation of the antitumor activity of fibroblast interferon. Information gained from the potentiator studies may lead to an increased efficacy of interferon treatment in the management of tumors. Similarly inhibitor studies may explain the need for the tremendous dosage levels of interferon required for tumor management and lead to a more effective interferon therapy. Further, studies of their roles in the modulation of the antiviral state and in the modulation of the immune response will be undertaken.

19. 2'-5'-LINKED OLIGONUCLEOTIDES OR GROWTH REGULATORS

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In recent years it has become quite evident that certain natural oligonucleotides exhibit growth regulatory function. Various oligonucleotides appear to regulate RNA polymerases and initiate cellular differentiation. Such an oligonucleotide pppA2'p5'A2'p5'A has recently been discovered in interferon treated cells and appears to be responsible for inhibition of cellular proliferation by regulating cellular messenger RNA synthesis. This trimer is active at subnanomolar levels and is not degraded by the usual ribonucleases due to the 2',5'-phosphodiester linkage. The aim of the present proposal is to synthesize such oligonucleotides, especially those containing a 2',5'-phosphodiester linkage and make such oligonucleotides available for antitumor evaluation in cell culture and in animal tumor model systems. It has been shown that certain oligonucleotides can enter tumor cells and it is hoped that such oligonucleotides will exert regulatory action which will control abnormal cellular proliferation characteristic of the cancer cell. Several different types of dinucleoside-2',5'-phosphate derivatives will be prepared and studied as inhibitors of DNA and RNA polymerase. Some monomeric derivatives such as tubercidin, toyocamycin and sangivamycin which exhibit definite antitumor activity in animal systems will also be studied as the 2',5'-linked oligomers with the hope that greater specificity of antitumor action might be obtained. Studies will be made of the synthesis and structure of certain oligonucleotide units structurally related to polyadenosine-diphosphate ribose (ADPR) which has been shown in eucaryotic cells to be obligatory for cell growth and division. The structure of poly ADPR has recently been shown to be that of a 2',1-alpha-D-O-ribofuranosyl-5-phosphate polymeric linkage (V). A synthesis of the monomer (1" yields 2') alpha-D-ribofuranosyladenosine-5',5"bis phosphate (V) and related analog derivatives is proposed. These oligonucleotides will be evaluated initially in an L-1210 cell culture system. Active oligonucleotides will then be synthesized in quantity sufficient for in vivo testing.

20. LYMPHOKINES FORMED IN VIVO - ROLE IN TUMOR SUPPRESSION

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Two lymphokines, migration inhibition factor (MIF) and immune interferon, appear in the serum of mice which have been sensitized to Mycobacterium bovis, strain BCG, and then challenged with specific mycobacterial antigens (old tuberculin). When this lymphokine-containing serum is injected into transplantatble murine tumors it markedly inhibits the growth of those tumors. This model will be used to evaluate the roles of MIF and immune interferon in tumor inhibition. The concentrations of MIF and interferon will be measured to determine if their activities parallel the tumor inhibiting activity of the serum.

Purification techniques including salt precipitation, column chromatography and isoelectric focusing will be used to attempt to enrich the factor(s) causing tumor regression and to determine if MIF and interferon activities are separable from the tumor inhibiting activity.

Studies will be made to determine physical-chemical characteristics of the tumor inhibitor such as molecular weight, heat stability and sensitivity to enzyme digestion. The mechanism of tumor rejection will be examined. Histologic studies will describe cells present at the tumor site. Inhibitors of lymphocyte and macrophage function will define cell populations required for tumor rejection. In vitro examination of the tumor recipient's antibody and lymphocyte response to tumor cells will show if the host's immune system is involved in the tumor rejection. It is hoped that these studies will help define the role these two lymphokine mediators play in tumor resistance.

21. REGULATION OF MAMMALIAN PROTEIN BIOSYNTHESIS

Clemens, M. J., Austin, S. A., University of London, St. Georges Hospital Medical School, Dept. of Biochemistry, *Cranmer Terr., Tooting, London SW17 0RE, England, United Kingdom*

OBJECTIVE: The aim of the project is to investigate several aspects of the regulation of protein synthesis in eukaryotic cells. It is proposed to analyze in depth the mecchanisms which are involved in control of translation at the cytoplasmic level in normal animal tissues and tumor cells grown in tissue culture. The modes of action of agents which affect cellular growth and division, including interferon and nutritional factors, will be studied.

APPROACH: The work will involve the preparation of rabbit reticulocytes and the growth of mammalian tumor cells in tissue culture. Cell-free extracts will also be prepared from these materials. Such systems will be used to study the mechanisms of action of interferon as both an anti-viral and an anti-cellular (anti-tumor) agent, and also the regulation of polypeptide chain initiation by variations in nutrient supply. The mode of action of haem in controlling protein synthesis in reticulocytes will also be investigated further.

Two complementary approaches will be used, as appropriate. In the first, attempts will be made to correlate the behavior of the intact cells with properties of the crude extracts derived

from them. In the second, individual components of the protein synthesizing machinery (such as initiation factors and mRNA) will be purified and used to reconstitute in vitro systems for assays of partial reactions of protein synthesis.

Other aspects of the control of gene expression and cellular growth will also be studied in attempts to define the detailed mechanisms of action of interferon and of essential nutrient supply; DNA synthesis, RNA synthesis and the transmembrane transport of the precursors for macromolecular synthesis will be investigated in their appropriate contexts.

PROGRESS: The results which constitute the basis of this project show that protein synthesis in mammalian cells is regulated at the translational level primarily at the stage of polypeptide chain initiation. Control of cellular growth rates by variations in nutrient supply (e.g. essential amino acids), and of protein synthesis in reticulocytes by haem, both involve modulation of formation of (40S ribosomal subunit. Methionyl-tRNA) initiation complexes. (Text Abridged.)

22. INDUCED DIFFERENTIATION OF ABELSON VIRUS-TRANSFORMED CELLS

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We have continued to study the differentiation capacity of Abelson murine leukemia virus-transformed hemopoietic cell lines. The cell line AT1 derived in vivo has now been analyzed. Similar to the in vitro derived line ABC-1 also described, it is composed of 1-4 percent cells with a pre-B lymphocyte phenotype with cytoplasmic IgM, but up to 35 percent of the cells can be induced to express this characteristic. Lipopolysaccharide, butyric acid, prostaglandin E1 and interferon were all found to be effective inducers. In contrast, dimethyl sulphoxide, phytohemagglutinin, dibutyryl cyclic AMP and hexamethylene bisacetamide were ineffective.

Induction was found to be independent of any growth rate reduction.

Incubation with lipopolysaccharide in the presence of the DNA synthesis inhibitors cytosine arabinoside or hydroxyurea, at doses that stopped growth, was permissive for induction. Therefore true induction was occurring rather than any selection and amplification of the pre-existing pre-B cell population. Whether this is induction of de novo mu chain transcription, translation and synthesis, or enhanced synthesis to detectable levels, is at present unknown.

Interestingly, both of the in vivo derived cell lines expressed lambda light chains. This indicates that the transformed cells are monoclonal and further suggests that the target cells may have undergone light chain restriction, in which case they would be considered B lineage committed cells.

23. INTERFERON SYSTEM - ACTION, INDUCTION AND REGULATION

Ts'o, P. O., Greene, J. J., Drocourt, J. L., Johns Hopkins University, School of Hygiene & Public Health, Dept. of Biochemistry, *615 N. Wolfe St., Baltimore, Maryland,* 21205, United States of America

This is a comprehensive study of interferon (IF) and double-stranded RNA (dsRNA) as a total system in both human and Syrian hamster cells. There are five major projects: (1) Function and structure relation of dsRNA as related to its involvement in the induction, action, and regulation of interferon and its intrinsic effects on cells. Extensive chemical modification of dsRNA and the use of anti-IF antibody are the strategy of this project. (2) The structure and function of 2'-5' oligo adenylic acid through the investigation of analogs. Analogs of 3'-deoxyribose and 3'-O-methylribose backbone have already been synthesized. The effects of these analogs on the 2'-5' oligo A activated reactions both in vitro and in vivo may provide unique insight into the role of 2'-5' oligo A in living cells. (3) Antineoplastic effects and antiproliferative effects of IF/dsRNA: Suppression of the expression of neoplastic phenotypes, suppression of progression in neoplastic transformation, and suppression of growth of normal versus tumorigenic cells will be investigated. Our very extensive experiences, accomplishments, facilities, and materials in in vitro carcinogenesis of hamster cells as well as in two human tumor cell lines will be the basis of this study. (4) The expression and influence of IF system in embryogenesis - Early results and literature reports indicate that the genes governing the IF system are being expressed during embryogenesis. This investigation of this differentiation system will provide insight on the relationship between various in vivo biological expressions and the in vitro biochemical reactions in the IF system, as the IF system is being developed from a dormant stage to an operational stage. (5) Somatic cell hybridization and somatic mutation studies of the IF system - In addition to the selection of variants/mutants resistant to the antineoplastic and antiproliferative effects of IF/dsRNA, somatic cell hybrids and mutants will be obtained for the studies on the IF regulation-shutoff systems. The research in the first year will be concentrated mainly on Projects 1, 2, and 3, and Project 4 and 5 will be investigated in subsequent years. Knowledge obtained from this research is vital for the use of IF as an antiviral and anticancer drug, and may also greatly enhance human IF production for practical use.

24. INTERFERON - MECHANISM OF ACTION

Brown, G., Levy, C. C., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Laboratory of Molecular Biology, *Baltimore, Maryland,* 21201, United States of America

Interferon has been shown to alter RNA metabolism by the induction of at least one endonuclease. Large changes in overall RNase activity are not normally induced by interferon. To extend these initial observations, we are looking at RNase and DNase activities in extracts derived from untreated and interferon treated cells using polynucleotide polyacrylamide activity gel electrophoresis. This system allows the detection and quantitation of individual activities rather than gross activity.

BIBLIOGRAPHIC REFERENCES: Brown, G.E., Simon, E.H. and Chung, C. Interferon production by individual L cells. J. Gen. Virol. 47: 171-182, 1980.

25. ANTITUMOR EFFECTS OF POLYRIBONUCLEOTIDES AND INTERFERON

Borden, E. C., McBain, J., Sack, K., U.S. Veterans Admin., Dept. of Medicine & Surgery, Medical Center, Oncology Service, *2500 Overlook Ter., Madison, Wisconsin,* 53705, United States of America

This work has the following objectives: 1) To further evaluate augmentation in biological activity of polyribonucleotides by polyene antibiotics, 2) to further define the effects of rIn-rCn and purified human fibroblast interferon in vitro on these cellular immune responses thought to be important in tumor immunity, 3) to clarify the antiproliferative effects of rIn:rCn and interferon on transformed and untransformed cells in vitro, and 4) to define the effects of interferons on papovavirus replication. This work is aimed at further defining the mechanisms of antitumor effects of interferon and the eventual therapeutic use of interferon and its inducers in neoplastic disease.

26. VIRUS-INDUCED ALTERATIONS IN ANIMAL CELLS

Tamm, I., Murphy, J. S., Gershey, E. L., Sehgal, P. B., Pfeffer, L. M., Wang, Y. E., Caliguiri, L. A., Rockefeller University, Virology Lab., *66th & York Ave., New York, New York,* 10021, United States of America

OBJECTIVES: 1) Identification, isolation, and characterization of initiation sites for DNA replication in mammalian cell DNA; 2) definition of the relationship between cell cycling and expression of SV40 virus gene function; 3) application of selective reversible inhibition of RNA polymerase II by DRB (5,6-dichloro-1-beta-D-ribofuranosylbenzimidazole) to studies of the synthesis of message-containing RNA of cells and viruses; and 4) elucidation of the action of interferon and DRB on cell proliferation.

These studies are designed to increase our understanding of the virus-cell interactions of simian virus 40 (SV40), adenovirus, and Rous sarcoma virus. The processes of cellular and viral DNA replication and RNA transcription, and of cell cycling will be investigated. The objective also is to define in detail the action of human fibroblast interferon on normal and transformed human cells. A wide variety of cellular biological, biochemical, and biophysical techniques will be used.

BIBLIOGRAPHIC REFERENCES: Sehgal, P.B. and Tamm, I. Antiviral agents: Determination of activity. In Antibiotics in Laboratory Medicine (Ed. V. Lorian). Williams and

Wilkins, Baltimore, Md., pp.573-591 (May, 1980). Sehgal, P.B. and Tamm, I. Regulation of interferon biosynthesis. In Report of the Second International Workshop on Interferon (M. Krim, W.E. Stewart II, V.G. Edy and H.S. Oettgen), The Rockefeller University Press, in press (1980).

27. THE ROLE OF INTERFERON IN ANTITUMOR IMMUNE RESPONSE

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We are investigating methods for the convenient production and purification of mouse immune interferon. We hope to obtain large amounts of this interferon free from contaminating lymphokine activities. This interferon will then be used to study the role of immune interferon in several antitumor immune responses.

We plan to investigate the role of purified immune interferon in augmenting the cytostatic and cytotoxic effects of macrophages on target tumor cells. These effects will be compared with those induced with fibroblast interferon. In addition, we will investigate the possible role of interferon-induced 2'-5' oligo(A) in macrophage-mediated cell killing.

Finally, we hope to determine whether immune interferon can substitute for lymphokine preparations during the activation of cytotoxic T lymphocytes by non-stimulator tumor cells.

28. ACTIVATION OF C-PARTICLES AND CANCER INDUCTION

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The Contractor will concentrate upon the action of human interferon as a prophylactic agent in reducing the incidence of significant viral infections and/or neoplasia following renal transplantation. Investigation of the activation of type-C oncornaviruses, BK papovaviruses or EW viruses following human renal transplantation. Investigation of the expression of type-C oncornaviruses and immune responses to these viruses in humans during normal gestation, oncogenesis or development of systemic lupus erythematosus. Studies of the interaction of primate type-C oncornaviruses or BK papovaviruses with human leukocytes in vitro. The molecular mechanisms underlying the activation of potentially oncogenic type-C oncornaviruses and papovaviruses.

29. MECHANISM OF ACTION OF INTERFERON

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No summary has been provided to the Smithsonian Science Information Exchange.

30. MECHANISM OF INTERFERON SYSTEM (MICE)

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This is part of a broader project. A summary of this subproject is not available.

C. ANTIVIRAL ACTIVITY OF INTERFERON

31. ISOLATION OF CLONALLY RELATED TRANSFORMED CELL LINES AND THE EFFECT OF INTERFERON ON VIRAL FUNCTIONS IN SUCH CELLS

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OBJECTIVE: To isolate a series of transformed cell lines obtained by infection of NIH-Swiss 3T3 cells with MLV/MSV and to study the effect of interferon on viral functions.

APPROACH: A series of transformed producer and non-producer lines of cells will be isolated and characterized for virus production (both MLV and MSV) and transformation phenotype. The effect of interferon on the production of MLV, MSV and the growth of normal and transformed cells will then be investigated.

PROGRESS: A series of producer and non-producer cell lines has been isolated and characterized. Interferon has been found to block replication by MLV at low doses and transformation by MSV at higher doses using both crude and purified mouse interferon.

32. ANTITUMOR EFFECT OF INTERFERON

Pitha, P. M., Raj, N. B., Riggin, C., Johns Hopkins University, School of Medicine, Dept. of Oncology, *725 N. Wolfe St., Baltimore, Maryland*, 21205, United States of America

Our studies of the effect of purified mouse interferon on the replication of MuLV in clonal cell lines indicated that interferon treatment inhibits virus replication in acute, endogenous and persistant infections. The major interferon block is not due to the general inhibition of protein synthesis but occurs at the level of virus maturation and assembly. In acute infection and MSV induced transformation (without helper) interferon, a small and reversible block of the early viral function was also detected. In cells persistantly infected with MuLV noninfectious virions are produced containing, in addition to the major structural proteins, uncleaved and partially cleaved precursors of both env and gag gene product.

It is our working hypothesis that the effect of interferon on MuLV assembly reflects and amplifies the anticellular effect of interferon (e.g., membrane effect) which is otherwise difficult to monitor on a biochemical level. Accordingly, we wish to examine and correlate 1) the molecular nature of MuLV inhibition and the anticellular activity of interferon, mainly its effect on mRNA population (cellular and viral) and its in vitro translatability effect on post-translational modification of proteins (e.g., glycosylation, phosphorylation) and rate of synthesis, and turnover of plasma membrane proteins and glycoproteins; 2) the effect of interferon on the physiological functions of plasma membrane and the membrane associated proteolytic activity.

We believe that the new knowledge gained through these studies will bring about important commentaries on the effect of interferon on retrovirus replication and its anticellular and antitumor activity, as well as a more precise scientific rationale for the therapeutic use of interferon.

BIBLIOGRAPHIC REFERENCES: Pitha, P.M., Fernie, B.F., Malderelli, F., Hattman, T. and Wivel, N.A., Effect of Interferon on MULV Infection V. Abnormal Proteins in Virions of Rauscher MULV Produced in the Presence of Interferon. J. Gen. Virol., in press, 1980. Pitha, Paula M., Fernie, B.F., Maldarelli, F., and Wivel, N.A., The Errors in Assembly of MULV in Interferon Treated Cells., in press, Jrnl. Supra. Struc., 1980.

33. PHARMACOLOGIC APPROACH TO AUTOIMMUNE (NZB) DESEASE

Buffett, R. F., Roswell Park Memorial Inst., *666 Elm St., Buffalo, New York*, 14263, United States of America

The objective of this proposal is to determine the effects of highly purified mouse interferon on the pathogenesis of autoimmune disease of New Zealand Black mice in which the ubiquitous presence of ecotropic and xenotropic murine leukemia viruses is considered to play a major role. The investigators will determine if the attenuation of the disease by interferon operates at the level of viral metabolism. Mice will be treated with highly purified mouse interferon (specific activity 2-3 times 10 to the 8th power units/mg of protein) for extended periods of time; treatment will be initiated at various times during the life of the animal, in relation to different stages in the progression of the disease. The effects of treatment will be measured by decreased levels of murine leukemia virus RNA-dependent DNA polymerase activity and gp69/71 antigen and virus antibody in sera and tissues, using competition radioimmunoassay, radioimmunoprecipitation and immunoelectrophoresis techniques. In addition, other parameters of the disease (development of antinuclear antibody and immunoglobulin, LE cells (lupus erythematosus cells) and other hematologic changes, etc.) will be monitored throughout life. The histopathologic status of interferon-treated mice will be evaluated by routine histologic and immunofluorescent microscopy of kidneys and other organs.

34. EFFECTS OF INTERFERON ON CELLS TRANSFORMED BY RNA TUMOR VIRUSES

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The major objective of this project is to define the effect of interferon on the expression of the sarcoma (src) gene of Rous sarcoma virus (RSV) in mammalian (rat) cells. Studies will be carried out to determine the effects of interferon on the rate of proliferation, the organization of actin-containing microfilaments, the distribution of cell surface fibronectin, and the level of plasminogen activator activity in RSV-transformed cells. Concurrently, experiments will be done to determine the rate of synthesis, membrane distribution, phosphorylation and enzyme activity of the src gene product which is a phosphoprotein with a molecular weight of 60,000 daltons (pp60). These experiments are designed to relate the effects of interferon on the transformed phenotype to its effect on src gene expression. Highly purified interferon will be used. Mouse interferon is active in rat cells. Two lines of rat cells will be used: a) RR1022, transformed by wild type Schmidt-Ruppin RSV; and b) LA25-NRK, transformed by a temperature-sensitive, transformation-defective mutant of Prague-A strain of RSV (LA25); this line will permit temperature shift experiments.

35. STUDIES OF MEASLES VIRUS OR OTHER VIRUS INFECTIONS

Gordon, I., University of Southern California, School of Medicine, Dept. of Microbiology, *2025 Zonal Ave., Los Angeles, California,* 90033, United States of America

We are studying the mechanism of action of different interferons (IFs). Principal emphasis is on the antiviral pathway; studies of the effects of IFs on cell or tumor growth will be limited to demonstrating that the antiviral and cytopathic pathways are disparate. We hope to identify the step or steps at which decay of IF antiviral action occurs during inhibition of the development of the antiviral state, and to continue the collection of data bearing on the interaction of IF and the cell membrane. Studies of the kinetics of synthesis of IF-mediated antiviral proteins are also planned.

BIBLIOGRAPHIC REFERENCES: Sakaguchi, A.Y., Stevenson, D., and Gordon, I. Inhibition of interferon production and antiviral action in mouse cells by 4,5',8-trimethylpsoralen activated with near ultraviolet light. Archives of Virology 61, 69-74 (1979). Gordon, I. and Stevenson, D. Kinetics of decay in the expression of interferon-dependent mRNAs responsible for resistance to virus. Proc. Natl. Acad. Sci. USA, 77, 4352, 1980.

36. FUNCTIONS OF INTERFERON-INDUCED 2',5'-OLIGOADENYLATE SYNTHETASE IN NORMAL AND VIRUS-INFECTED CELLS

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Interferon is a protein made by cells in response to viral infection. The protein functions to protect cells from viral infection by interfering with virus replication. Interferon has shown great promise in clinical trials for the treatment of patients with cancer or with virus infections. It is now becoming clear how interferon may specifically disrupt virus-infected or cancer cells without affecting normal cells. Dr. I.M. Kerr and associates made an important breakthrough when they discovered that interferon induces the synthesis of 2',5'-oligoadenylates (abbreviated 2-5A). These oligoadenylates function by inhibiting protein synthesis. The work proposed in this application, some of which will be done in Dr. Kerr's laboratory, is aimed at understanding how 2-5A specifically disrupts viral protein synthesis and also how 2-5A might function in normal cells. This will be done by completing the purification of 2-5A synthetase, an enzyme induced by interferon and responsible for making 2-5A. The enzyme purification will be done in the laboratory of L. Andrew Ball (Madison), a former associate of I.M. Kerr. Antisera against the synthetase will be used for the isolation and purification of synthetase mRNA, for detection of the enzyme in normal cells at various growth stages and of different hormone status, and, if possible, to determine the cellular localization of the synthetase. The synthetase makes 2-5A by catalyzing the addition of adenylate residues (one to four) to the 2'-hydroxyl group of an ATP primer. Dr. Ball has found that several other adenine metabolites such as NAD(plus) and AppppA can function as primers. It is possible that one or more of these 2'-oligoadenylate derivatives will have profound effects on cells since NAD(plus) and AppppA have been implicated in the control of DNA synthesis. These substances will be tested in permeabilized whole cells and, if appropriate, in cell-free extracts. Attempts will also be made to develop oligoadenylate affinity columns for the isolation of proteins that control viral protein synthesis.

37. DIFFERENTIAL EFFECTS OF INTERFERON ON PRE-REPLICATIVE BIOCHEMICAL EVENTS ELICITED BY SERUM OR BY DEFINED GROWTH FACTORS IN 3T3 CELLS

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The metabolic events that occur after mitogenic stimulation of quiescent fibroblasts have been divided into 'EARLY' changes that are independent of protein synthesis (ion fluxes, nutrient uptake, etc.) and 'LATE' events, which are dependent on the de novo synthesis of proteins (uptake systems induction of enzymes). We have examined whether any of these pre-replicative events in serum-stimulated quiescent 3T3 cells are affected by mouse interferon. Our results show that interferon does not inhibit the early events such as the uptake of 2-deoxyglucose, uridine, phosphate, rubidium. However, a differential inhibitory effect on protein synthesis-dependent events is observed. Thus the large increase in the synthesis of ornithine decarboxylase (CDC) activity observed in stimulated cells is inhibited while the second phase of 2-deoxyglucose transport and total protein and RNA synthesis is unaffected. Pure growth factors induce a higher level of ODC than serum and again interferon is effective in selectively inhibiting the induction of enzyme activity. Interferon appears to be selectively inhibiting the synthesis of specific proteins; this is indicated by comparison of the electrophoretic patterns of the 35S-methionine-labelled proteins, synthesized in quiescent cells stimulated by serum or growth factors, in the presence and absence of interferon. Experiments using metabolic inhibitors indicate that inhibition of enzyme induction may be occurring at both the transcriptional and translational level.

38. MODE OF ACTION OF INTERFERON - MECHANISMS OF THE INDUCTION AND ACTIVITY OF THE INTRACELLULAR ANTIVIRAL STATE

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OBJECTIVE: To understand the molecular mechanisms in the induction and activity of the antiviral state by interferon.

APPROACH: Identification, characterization and subcellular localization of new proteins made in response to treatment with interferon. Structural analysis of defective virions produced by cells treated with interferon.

39. REPLICATION OF VIRULENT AND ONCOGENIC VIRUSES

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The overall objective of this research is to determine the role of differential initiation rates on mRNAs in regulating protein synthesis in normal, virus-infected, interferon treated and transformed cells. The significance of the inhibition of the rate of translation elongation in interferon-treated cells will be assessed, especially in regard to its possible selectivity against rapidly initiating (viral) mRNAs. The mechanism by which elongation inhibition is accomplished will also be studied. The intrinsic initiation rates on various viral and host mRNAs will be measured, and the factors which determine intrinsic initiation rate will be identified. Particular attention will be paid to the role of mRNA 'cap' structures, and cap-specific initiation factors.

BIBLIOGRAPHIC REFERENCES: Characterization of the Proteins of Intracisternal Type A and Extracellular Oncornavirus-like Particles Produced by MOPC-460 Myeloma Cells, by Donald L. Robertson, Perin S. Jhabvala, Therese Godefroy-Colburn and Robert E. Thach. J. Viral., 32, 114-122 (1979). Characterization of Infectious Oncornaviruses from MOPC-460

Plasmacytomas: Their Relation to A-Type Particles, by Triprayar V. Ramabhadaran, Janet W. Hartley, Wallace P. Rowe, Therese Godefroy-Colburn, Perin S. Jhabvala and Robert E. Thach. J. Virol., 32, 123-130 (1979).

40. VIRUS- AND CELL-INHIBITORY ACTIVITY OF INTERFERON

Joklik, W. K., Cashdollar, W. L., Stone, H. O., Morgan, J. H., Graff, J. C., Duke University, School of Medicine, Dept. of Microbiology & Immunology, *Box 3711, Durham, North Carolina*, 27710, United States of America

Work is proposed on three projects involving interferon. First, we wish to determine whether reduction of the stability (that is, the half-life) of viral messenger RNAs plays a role in the mechanism of antiviral action of interferon. Specifically, we wish to measure the half-life of vaccinia virus, VSV and EMC virus messenger RNAs in normal and interferon-treated cells. Second, we will continue our studies to clone a mouse interferon gene. Once such a gene has been cloned, the mouse interferon DNA will be used to isolate pure messenger RNA of the homologous human interferon gene, which will then be cloned in vectors and under conditions that are most likely to lead to its complete expression. Finally, we propose a series of studies to isolate human cells capable of producing interferon constitutively. Cells will be mutagenized, and a variety of human cell lines will be surveyed for their ability to yield constitutive interferon producers by selecting those able to resist infection with virus.

41. CHARACTERIZATION OF INTERFERONS AND THEIR ACTIONS

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The proposed studies will define the various molecular forms of human and murine type I and type II interferons in terms of the extent of physicochemical and biologically detectable variations. By correlating these variations with their abilities to exert diverse antiviral, antitumor and non-antiviral activities in homologous and heterologous cells, we propose to define structure-function relationships of the interferons. By isolation of interferon messenger RNAs from the various induced cells producing distinguishable human interferons or distinguishable mouse interferons and by translating these mRNAs in various cellular and cell-free systems under conditions either promoting or restricting post-translational modifications, we propose to isolate modified interferons whose physicochemical and biological (antiviral:non-antiviral activity ratios and host specificities) properties can be compared to native interferons, potentially enabling production of interferons with selected properties. We propose also to chemically or enzymatically degrade native or modified interferons to produce small 'active-core' fragments which can themselves be characterized, to detect differences between those and native interferons in terms of physicochemical and biological properties. Studies on the affinities of the distinct forms of native interferons and of modified or fragmented interferons for various tissues are proposed to determine the physiological significance of distinct interferon forms.

BIBLIOGRAPHIC REFERENCES: Lin, L.S., Wiranowska-Stewart, M., Chudzio, T. and Stewart II, W.E. (1978). Characterization of the size and charge heterogeneities of human leukocyte interferon populations. Arch. Virol. 56, 269-272. Stewart II, W.E. (1978). The Interferon System, Springer-Verlag, Vienna, in press.

42. MOLECULAR MECHANISMS OF ANTIVIRAL RESISTANCE

Grossberg, S. E., Mehler, A. H., Sela, I., Sedmak, J. J., Chakraburtty, K., Keay, S., Medical College of Wisconsin, School of Medicine, Dept. of Microbiology, *561 N. 15th St., Milwaukee, Wisconsin*, 53233, United States of America

Our long-range objective is to determine the molecular basis for antiviral resistance. We have recently discovered a factor that interferes with the aminoacylation of viral-RNA but does not appear to affect the normal cellular cognate tRNA; more specifically, this factor appears to cause the discharge of amino acid that has already been esterified to the viral RNA. Using this in vitro phenomenon as an assay we intend to (1) purify the discharging factor(s), (2) characterize the elements of the in vitro reaction, and (3) study the biology of the phenomenon in relation to what is currently known about (a) interferon-stimulated antiviral resistance, (b) antiviral resistance not necessarily related to interferon, and (c) non-antiviral effects of interferon, such as inhibition of cell growth. The biology of the factor(s) will be studied, as appropriate, in normal diploid cells, in cells with chromosomal abnormalities that enhance or depress the response to interferon, cells that are sensitive or resistant to interferon, including cancer cells (especially melanoma lines and carcinoma lines from bladder and lung), and embryonic cells, with regard to uptake, activity, and distribution in intact cells, kinetics of appearance, dose-response relationships, and effects of metabolic or natural tissue inhibitors. The investigations proposed are a detailed analysis of the phenomenon and its possible relationship to several biological phenomena involving natural and induced resistance to viruses of plants, animals, and human beings.

BIBLIOGRAPHIC REFERENCES: Grossberg, S.E.: Interferon Standards: A Memorandum (report to the World Health Organization on a workshop on interferon standards, September 1978). Journal of Biological Standardization, 1979, in press. Jameson, P., Greiff, D., and Grossberg, S.E.: Thermal Stability of Freeze-Dried Mammalian Interferons: Analysis of freeze-drying conditions and accelerated storage tests for murine interferon. Cryobiology, 1979, in press.

43. RIBONUCLEASE ACTIVITY OF PREPARATIONS OF HUMAN LYMPHOBLASTOID INTERFERON

Karpetsky, T., Brown, G., Biswal, N., Levy, C. C., Bandyopadhyay, A., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Laboratory of Molecular Biology, *Baltimore, Maryland*, 21201, United States of America

Crude human lymphoblastoid interferon (IF) has less ribonuclease (RNase) activity than equivalent primary leukocyte IF. RNase activity was abolished when the IF was purified by methods that differed from those that had failed to eliminate similar activity from leukocyte IF. It is thought unlikely that exogenous RNase plays a major role in the antiviral action of IF preparations. Basic ribonuclease activity, rather than simply the presence of protein could be detected using polynucleotide-polyacrylamide gel electrophoresis. Polyacrylamide gels were prepared containing low concentrations of a polynucleotide substrate. Spermine, present in high amounts during electrophoresis, enables the basic RNases to migrate in the presence of polyanionic substrates. Electrophoresis of the RNases and incubation of the gel at neutral pH followed by staining results in a colored gel containing clear bands which define regions of enzyme activity. Higher resolution of activity regions was achieved by using polyacrylamide gel electrophoresis in one direction, followed by polynucleotide-polyacrylamide gel electrophoresis in a direction perpendicular to the first migration. Enzyme activities are visualized as coloriess spots on a purple slab. Preliminary results also indicate that pretreatment leukemia patients have an additional lymphocyte nuclease activity when compared to lymphocyte activity patterns obtained from normal volunteers.

BIBLIOGRAPHIC REFERENCES: Davies, G.E., Karpetsky, T.P. and Levy, C.C.: The ribonucleases of bovine skeletal muscle. Biochem. J.: in press, 1980. Karpetsky, T.P., Davies, G.E., Shriver, K.K. and Levy, C.C.: Use of polynucleotide-polyacrylamide-gel electrophoresis as a sensitive technique for the detection and comparison of ribonuclease activities. Biochem. J.: in press, 1980.

44. INTERFERON - MECHANISM OF ACTION

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This is part of a broader project. A summary of this subproject is not available.

45. MECHANISM OF INTERFERON SYSTEM (MICE)

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The summary for this project is under entry no. 30.

46. MECHANISM OF ACTION OF INTERFERON

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The summary for this project is under entry no. 29.

D. INTERFERON AND IMMUNOREGULATION

47. CONFERENCE ON REGULATORY FUNCTIONS OF INTERFERONS

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We are proposing that a Conference entitled 'Regulatory Functions of Interferons' be held under the sponsorship of the New York Academy of Sciences. Interferons are known to exert a multitude of biological effects. Although the antiviral actions have been most thoroughly studied, the modulation of cellular susceptibility to virus infection is not the only important function of interferons. The idea that interferons act as immunoregulatory substances has now gained broad acceptance. The known effects of interferons on immune functions include the following: modulation of antibody response, inhibition of the mitogenic response of lymphocytes, activation of macrophages, enhancement of killer T cell activity and enhancement of the activity of a special population of lymphocytes termed 'natural killer' (NK) cells. Two distinct types of 'conventional' interferons are made by various cell types. In addition, distinct interferon activity is produced by sensitized T cells in response to specific antigen ('immune' or 'type II' interferon) and it appears that this type of interferon (a lymphokine) is a more active immunoregulator than conventional interferons. Other regulatory effects of interferons include: effect on expression of cell surface antigens, inhibitory action on cell division and tumor growth, inhibition of intracellular microorganisms (eg., chlamydia, rickettsia, protozoa). The potential of interferons as antitumor agents is now being evaluated in controlled clinical trials sponsored by N.C.I. and the American Cancer Society. Progress has recently been made in the elucidation of interferon action at the molecular level; these studies unexpectedly revealed the existence of molecular mechanisms of possible broad regulatory significance. The role of the proposed Conference would be to provide a coherent picture of the different regulatory functions of various interferons. The stress would be on interdisciplinary aspects that would be of interest to a broad audience of immunologists, cell biologists, molecular biologists and virologists.

48. CONTROL OF NEOPLASTIC DISEASES BY COMBINED TREATMENT WITH IMMUNO-REGULATING FACTORS

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Several agents were found to enhance macrophage and natural killer cell tumoricidal activity (poly ICLC, lentinan, thymopoeitin fractions, MVE, glucan and human chorionic gonadotropin). The enhanced activity was neutralized by interferon antibody, indicating that interferon is the direct activating factor. The tumoricidal activity of interferon may be due to its capacity to enhance macrophage and natural killer cell reactivity to tumor antigens. Prostaglandins E1 and E2 negate macrophage tumoricidal activity via 'shut off' mechanism. TPA, a potent inflammatory and tumor-promoting agent possesses two opposing influences on macrophage reactivity. Conditioned medium from macrophages pulsed with TPA enhanced M109 carcinoma colony formation in vitro. Splenocytes incubated with TPA produced macrophage activating factor. Human chorionic gonadotropin enhanced macrophage and natural killer activity. Human monocyte cultures exposed to human interferon exerted a stronger cytotoxic effect on the Raji lymphoma tumor cell line. Significant adjuvant effect was attained with MVE when it was combined with B16 melanoma vaccine. Increased survival was attained in L1210 leukemia when Azimexon (BM 12.531) and MVE were combined with chemotherapy.

BIBLIOGRAPHIC REFERENCES: Schultz, R. M., and Chirigos, M. A.: Macrophage Activation for Non-Specific Tumor Cytotoxicity: A Review. In Schnitzer, R. J. (Ed.): Advances in Pharmacology and Chemotherapy - Vol. 17. New York, Academic Press, 1980 (in press). Schultz, R. M., Chirigos, M. A., and Olkawski, Z. L.: Stimulation and inhibition of neoplastic cell growth by tumor promoter-treated macrophages. Cell. Immunol. 1980 (in press).

49. C-REACTIVE PROTEIN REGULATION OF TUMOR IMMUNITY

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The interaction between the inflammatory and immune systems has traditionally focused on the action of activated complement proteins and lymphokines on the cells mediating inflammation; by contrast, we will examine the influence of the acute inflammatory reactant, C-reactive protein (CRP), on the immune functions of lymphoid cells. Although elevated levels of CRP accompany a wide variety of diseases, its biological role in host defense is still unknown. To delineate the effects of CRP on immune functions we will use the T-cell specific binding property of CRP to isolate CRP-binding cells and characterize their surface antigens and receptors. Analysis of the influence of CRP on anti-tumor immune reactivities stems from our observations of the activation of nonspecific suppressor mouse T-cells and will include tests of the ability of CRP to limit not only sensitization to tumor antigens, but also the expression of tumor specific immune reactivity in vitro and in vivo. Tumor specific cell-mediated immune reactivity of breast cancer patients and mice will be studied to determine the cellular mechanism of suppression by CRP. Binding of CRP to lymphoid cells that have infiltrated regressing and progressing animal tumors will be measured. CRP will be compared to interferon and interferon inducers both as an activator of macrophages to the tumoricidal level and as an augmentor of NK cell activity. The perspective for comparison with interferon is the analogous induction of both substances. Eventually we hope to elucidate ways by which CRP can be manipulated clinically to the advantage of the host and its serum levels interpreted in a meaningful way.

50. NATURAL AND TISSUE-SPECIFIC IMMUNITY TO HUMAN NEOPLASMS

Saksela, E. J., Wahlstrom, T. R., Timonen, T., Paasivuo, R., Helsingin Yliopisto, *Fabianinkatu 33, SF00290 Helsinki, Finland*

Methods have been developed for enrichment and characterization for human natural killer (NK) cells. The morphology, functional characteristics and certain surface characteristics of human NK cells have been described.

The effect of interferon on the fractionated human NK cells has been studied. We have demonstrated that NK cells produce interferon when contacted with tumor target cells and the produced interferon recruits an initially non-cytoxic 'pre-NK' cell population to killer cells with the properties of NK cells. There is thus, within the NK system itself a self-amplifying positive feed-back mechanism which may be potentially important as a first line of defence against tumor cells.

Using the morphology of the human NK cells as markers we have improved the fractionation methods and obtain 85-90 percent pure fractions of such cells for continuation studies.

The presence of NK cells within human solid tumors has been investigated and the program on the specific immunological characteristics of normal and neoplastic glial cells continued. Various other tumor markers have been investigated and also applied to clinical diagnostic work.

BIBLIOGRAPHIC REFERENCES: Timonen, T., Ranki, A., Hayry, P. and Saksela, E.: Human natural cell-mediated cytotoxicity against fetal fibroblasts. III. Morphological and functional characterization of the effector cells. Cellular Immunol. 48: 121-132, 1979. Timonen, T., Saksela, E., Ranki, A.-M. and Hayry, P.: Fractionation morphological and functional characterizaton of effector cells responsible for human natural killer activity against cell line targets. Cellular Immunol. 48: 133-148, 1979.

51. CONTINUED STUDIES ON THE REGULATION OF HUMORAL AND CELLULAR IMMUNE FUNCTIONS

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OBJECTIVE: To gain a deeper understanding of the immune mechanisms that regulate resistance to tumor cells as well as conventional antigens.

APPROACH: Study tumor-immune reactions in vitro and in vivo with regard to NK cells and specific T lymphocytes using procedures allowing the selective increase or decrease of subsets of specific cells. Define the surface structures on lymphoid normal and malignant cells. Clone specific lymphoid cells.

PROGRESS: NK cells can be induced by interferon and maintained in enhanced activity in vitro or in vivo for prolonged periods of time. Specific T cell receptors have been isolated and found to express significant although weak reactivity for self-MHC in relation to strong reactivity towards allo-MHC. NK susceptibility of fresh human lymphoma cells has been demonstrated. A unique lectin, reactive selectively with cytolytic T cells, has been discovered. Alpha-fetoprotein acts as a selective blocker of macrophage-dependent, interferon-dependent activation of NK cells.

52. PSYCHOLOGICAL FACTORS AS RELATED TO INTERFERON AND NATURAL KILLER CELL ACTIVITY

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Earlier findings suggest that there is a relationship between self-reported psychiatric symptoms and natural killer cell (NK) activity. Further work demonstrated that the self-reports to a considerable degree reflected trait chracteristics in psychological tests. In an effort to determine whether interferon might have stimulated the appearance of the NK activity of those with high levels, an interferon analysis of the stored sera will be carried out.

53. CELLULAR IMMUNE RESPONSES IN EXPERIMENTAL TUMOR SYSTEMS AND TUMOR PATIENTS

Herberman, R. B., Korec, S. M., Nunn Hargrove, M. L., Ortaldo, J. R., Reynolds, C. W., Timonen, T., Goldfarb, R., Gorelik, E., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Laboratory of Immunodiagnosis, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

Human and rat natural killer (NK) cells and K cells mediating antibody-dependent cellular cytotoxicity have been shown to be large granular lymphocytes (LGL). The majority of LGL formed conjugates with, and caused lysis of, NK-susceptible target cells. The specificity of human NK cells was examined by selective monolayer adsorptions and evidence for multiple antigens, recognized by subsets of NK cells, was obtained. Interferon caused direct augmentation of NK and K cell activities of LGL, with the predominant mechanism being an increase in lytic efficiency. Studies on mechanisms for depressed NK activity have shown: suppressor cells in normal SJL mice; patients with Chediak-Higashi syndrome to have a marked, selective deficit in NK and K cell activities; and the carcinogen, urethane, and the tumor promotor, phorbol ester, to be able to depress NK activity. In short-term in vivo assays, NK cells played a major role in resistance to tumor challenge. Granulocytes of normal human donors have substantial cytostatic activity against cultured cell lines and this activity was depressed in most tumor-bearing patients, apparently due to circulating inhibitory factors. Lymphoproliferative response of breast cancer patients to autologous tumor antigen was shown to be a significant prognostic factor, with lack of activity predictive of disease recurrence.

BIBLIOGRAPHIC REFERENCES: Haliotis, R., Roder, J., Klein, M., Ortaldo, J., Fauci, A.S., and Herberman, R.B.: Chediak-Higashi gene in humans. I. Impairment of natural-killer function. J. Exp. Med. 151: 1039-1048, 1980. Santoni, A., Riccardi, C., Sorci, V., and Herberman, R.B.: Effects of adriamycin on the activity of mouse natural cells. J. Immunol. 124: 2329-2335, 1980.

54. CELL-MEDIATED CYTOTOXICITY IN HUMANS

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The identification and quantitation of human cytotoxic cells are basic for the understanding of the immune response to allo-antigens, intracellular infectious agents and tumor antigens, and might acquire clinical relevance for the evaluation of the patient's immune status. We propose to investigate some aspects of cell-mediated cytotoxicity in humans: 1) To analyze the phenomenon of interferon induction in mixed cultures of lymphocytes and virus infected or tumor-derived cells; 2) to investigate the enhancing effect of interferon and interferon inducers on natural killer cells; 3) to study the mechanism of interferon-induced resistance to lysis of target cells; 4) to identify cytotoxic cell types generated during an immune response; 5) to analyze the specificity and the genetic control of cytotoxic T cells against virus infected and transformed cells; 6) to produce by somatic cell hybridization monoclonal antibodies against lymphocyte surface antigens able to identify lymphocyte subpopulations.

55. INTERFERON - EFFECT ON IMMUNITY TO BLADDER TUMORS

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The objective of our proposed research is to define the effect of Type II interferon on the cell-mediated immune response to bladder tumors and compare it with the effect of Type I interferon. To this end we will test the effect of interferon (both Type I and Type II) treatment in vitro on human mononuclear cells isolated from the peripheral blood for: (1) Effect on antibody-dependent cell-mediated cytotoxicity using 253J human bladder cancer cells, (2) effect on natural killer cell activity using K562 human lymphoma and 253J target cells, (3) effect on the generation specific T cell mediated cytotoxicity against 253J target cells. In addition, a murine animal model system will be developed using the FANFT-induced bladder tumor, MBT-2, to evaluate the effect of interferon (Type I and Type II) in vivo and in vitro on the cell-mediated cytotoxic mechanisms listed above using appropriate mouse target cells including MBT-2 cells. Moreover, the effect of interferon-induced modulation of the immune response on the growth of transplanted MBT-2 bladder tumors in syngeneic mice will be evaluated.

We will determine the dose and class (Type I or Type II) of interferon most effective for modulation of the cell-mediated responses in vitro for both the human and murine models and in vivo for the murine model. Moreover, we will determine the most effective dose and class of interferon (Type I or Type II) for prevention of the growth of transplanted MBT-2 tumors in syngeneic mice.

We will determine the effect of interferon (Type I and Type II) on the induction of primary murine bladder tumors by FANFT.

56. CELLULAR IMMUNITY TO TUMORS AND HERPES INFECTIONS

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The research proposed for the remaining four months of this grant is directed at continuing our studies aimed at generating cytotoxic lymphocytes in vitro, capable of lysing autologous human leukemia and lymphoma cells. Attempts will be made to generate cytotoxic T lymphocytes (CTLs) in mixed leukocyte cultures (MLCs) by culturing remission T cells with a) x-irradiated autologous leukemia cells and x-irradiated allogeneic stimulating cells in 'three-cell' protocols or with b) x-irradiated lymphocytes pooled from 20 unrelated normal individuals. The addition to these MLCs of T cell growth factor (TCGF), highly purified human fibroblast interferon (HFIF) or polyribonucleotide inducers of interferon (IF) may augment the generation of CTLs to autologous malignant cells since these agents markedly

enhance the generation of CTLs to alloantigens. A main goal of this research will be to generate large numbers of CTLs cytotoxic for autologous malignant cells; thus, continuous cultures of CTLs, cytotoxic for autologous malignant cells, will be established in TCGF. Further, we propose to begin to isolate clones of CTLs, cytotoxic for autologous malignant cells, with which to determine whether malignant cells from different patients express common target antigens for CTLs.

57. CELLULAR IMMUNITY TO TUMORS

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The proposed research is a continuation of our studies aimed at generating cytotoxic lymphocytes in vitro, capable of lysing autologous human leukemia and lymphoma cells. Attempts will be made to generate cytotoxic T lymphocytes (CTLs) in mixed leukocyte cultures (MLCs) by culturing remisson T cells with a) x-irradiated autologous leukemia cells and x-irradiated allogeneic stimulating cells in 'three-cell' protocols, or with b) x-irradiated lymphocytes pooled from 20 unrelated normal individuals. The addition to these MLCs of T cell growth factor (TCGF), highly purified human fibroblast interferon (HFIF) or polyribonucleotide inducers of interferon (IF) may augment the generation of CTLs to autologous malignant cells since these agents markedly enhance the generation of CTLs to alloantigens. A main goal of this research will be to generate large numbers of CTLs cytotoxic for autologous malignant cells; thus, continuous cultures of CTLs, cytotoxic for autologous malignant cells, will be established in TCGF. Further, we propose to isolate clones of CTLs cytotoxic for analogous malignant cells and to use these monoclonal CTSs to determine whether malignant cells from different patients express common target antigens for CTLs. As a correlate to the in vitro human studies, mouse AKR CTLs grown in TCGF will be tested for their ability to prolong survival of AKR leukemic mice following chemoradiotherapy. HFIF, polyinosinic: polycytidylic acid (poly I:C) and analogues of poly I:C (which differ in their ability to induce IF and cause toxic effects) that augment human natural killer (NK) cell activity against leukemia cell lines will be tested for their ability to activate NK cells cytotoxic for fresh autologous leukemia and lymphoma cells. Poly I:C and nontoxic analogues of poly I:C will also be tested in mice for their ability to activate NK cells cytotoxic for syngeneic leukemia cells and for their ability to prolong survival of AKR leukemic mice.

58. ANTITUMOR EFFECTS OF POLYRIBONUCLEOTIDES AND INTERFERON

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The summary for this project is under entry no. 25.

59. STUDIES OF THE DEFENSE AGAINST HERPES SIMPLEX VIRUS

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Antiviral defense mechanisms and the cellular immune response are studied in a mouse model of HSV infection. In this model inbred strains of mice resistant or susceptible to HSV and pathogenic and apathogenic virus strains are compared. Recently we have also compared HSV type 1 and HSV type 2. The specific cell-mediated immune response is measured by lymphoproliferation or by lymphokine production (migration inhibitory factor, immune interferon). In studies of nonspecific defense we are investigating the role of macrophages, of natural killer cells, and of interferon. We are particularly interested in the effects of HSV on cellular immunity (replication of HSV in leucoytes, immunosuppression by HSV). Selected experiments will be performed using human peripheral lymphocytes.

60. IMMUNE INTERFERON IN CELLULAR, VIRAL AND IMMUNE SYSTEMS

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No summary has been provided to the Smithsonian Science Information Exchange.

61. EFFECT OF INTERFERON AND INTERFERON INDUCERS ON CELL IMMUNE RESPONSE

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This is part of a broader project. A summary of this subproject is not available.

E. PURIFICATION AND BIOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF INTERFERON

62. PURIFICATION AND IMMUNOLOGICAL/ BIOLOGICAL/BIOCHEMICAL CHARACTERIZATION OF HUMAN LEUKOCYTE INTERFERONS

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OBJECTIVE: The main purpose is to purify all species of human leukocyte interferon and to examine the varied biological functions of the individual species in vitro and in vivo with regard to a) antiviral activity, b) anti-cellular activity, c) potentiation of the NK system, d) potentiation of the MLC-CML system, e) potentiation of the expression of HLA antigens, and f) potentiation of the expression of DR antigens.

APPROACH AND PROGRESS: The human leukocyte interferon has now been completely purified (Scand. J. Immunol. 11, 489-502, 1980); two major species and three minor species have been demonstrated. All the species are currently being examined in the above mentioned systems.

63. CHARACTERIZATION OF HUMAN NATURAL KILLER CELLS

Tarkkanen, J., Saksela, E., Timonen, T., Helsingin Yliopisto, Dept. of Pathology, *Fabianinkatu 33, SF00290 Helsinki, Finland*

OBJECTIVE: Human natural killer cells (NK-cells) and interferon are currently under worldwide laboratory investigation. This results from their possible role in the genesis and treatment of cancer. We will study the NK-cells of both healthy donors and cancer patients, and examine the problem of how cancer cells escape surveillance by the immune system in vivo.

APPROACH: We have developed techniques in our laboratory to isolate human NK-cell, taking advantage of the fact that NK-cells adhere to the surface of sensitive target cell (Timonen et al.). Utilizing this adsorption-elution technique we will further characterize the properties of NK cells. We have demonstrated that NK-cells display autologous restriction. This can, however, be abolished through contact with cancer cells (Tarkkanen et al.). We are currently studying the effect of interferon on these aspects. We will also initiate a project with cancer patients to determine whether the reactivity of their NK-cells differs from that in healthy donors.

64. CHARACTERIZATION OF HUMAN INTERFERON

Vilcek, J. T., Pang, R. H., Yip, Y. K., New York University, School of Medicine, Dept. of Microbiology, *550 1st Ave., New York, New York,* 10016, United States of America

Work will be continued on the development of methods for the production and purification of human type II (immune) interferon. Production will be scaled up by the use of buffy coats from the blood bank instead of fresh blood. A number of purification steps will be evaluated, including chromatography on controlled pore glass, blue Sepharose, Con-A Sepharose, and gel filtration of Biogel P-150. Purified Le, F and T interferons will be compared in their biological activities including antiviral action, growth inhibitory action, activation of natural killer (NK) cell activity, and induction of 2'5' (pA)n synthetase activity.

BIBLIOGRAPHIC REFERENCES: J. Vilcek, I.T. Sulea, F. Volvovitz, and Y.K. Yip: Characteristics of interferons produced in cultures of human lymphocytes by stimulation with Corynebacterium parvum and phytohemagglutinin, p. 323-329. In: Biochemical Characterization of Lymphokines, Academic Press, Inc. (1980). G. Klein and J. Vilcek: Attempts to induce interferon production by IdUrd-induction and EBV superinfec-

tion in human lymphoma lines and their hybrids. J. Gen. Virol. 46: 111-117 (1980).

65. CHARACTERIZATION OF INTERFERONS AND THEIR ACTIONS

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The summary for this project is under entry no. 41.

66. TO STUDY RELATIONSHIP BETWEEN STRUCTURE INTERFERONS AND THE DIVERSE ACTIVITIES

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This is part of a broader project. A summary of this subproject is not available.

67. INTERFERON STRUCTURE AND FUNCTION (MICE)

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This is part of a broader project. A summary of this subproject is not available.

68. INTERFERON AS A MEDIATOR OF CELLULAR IMMUNITY

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Recent evidence has indicated that there is heterogeneity among interferons produced by different cells responding to a variety of stimuli. Thus far very little has been done to characterize these interferons and to examine their functional interrelationships. Therefore we plan to examine the synthesis of interferon by subsets of human T lymphocytes prepared by immunologic fractionation (T gamma-T mu-T phi) and by density gradient fractionation in response to mitogens (PHA, PWM, Con A), C. parvum, bacterial antigens (PPD) and viral antigens (vaccinia).

We will also characterize the physical properties and host range specificity of immune interferons and determine if these properties are influenced by the nature of the inducer, by the cell of origin, or by the accessory cells in culture, and we will compare immune and classical interferons.

Furthermore, since interferon is itself produced by cells of the immune system its synthesis reflects the status of that system and can now be used as a means to dissect out abnormalities of lymphocyte, monocyte, and macrophage function in various disease states. We will therefore study in patients with immunodeficiency diseases (selective IgA deficiency, ataxia-telangiectasia and thymic hypoplasia) and thymic hypoplasia) and autoimmune diseases (lupus erythematosus) the production of immune interferon by mitogen or C. parvum stimulated lymphocytes as a measure of the effector competence of T lymphocyte subsets and as a measure of the integrity of macrophage function.

BIBLIOGRAPHIC REFERENCES: Epstein, L.B. Mitogen and antigen induction of interferon in vitro and in vivo, Texas Reports of Biol. and Med., 1978, in press. Yatsiv, S., Epstein, L.B., and Epstein, C.V., Monocyte-derived macrophages: an in vitro system for studying hereditary lysosomal storage diseases, Pediatric Research, 1978, in press.

69. HUMAN FIBROBLAST INTERFERON

Tan, Y. H., Huang, R. C., University of Calgary, School of Medicine, Division of Medical Biochemistry, *26th St. & 26th Ave. N.W., Calgary, Alberta*, T2N 1N4, Canada

The purpose of this project is to consolidate our recent observations made on the molecular events following the induction of the human interferon gene(s) in cell line C-10 by poly r I poly r C. Specifically we are concerned with: (1) the changes in cellullar RNA metabolism at the level of its messenger RNA during induction; (2) the changes in cellular nucleases coordinated with changes in its cellular RNA during induction; and (3) the purfication and isolation of the messenger RNA for human fibroblastoid interferon.

70. SOMATIC CELL GENETIC STUDIES OF THE INTERFERON SYSTEM

Veomett, G. E., University of Nebraska, Lincoln Campus, School of Life Sciences, *Lincoln, Nebraska*, 68508, United States of America

We propose to study the control of the interferon system in mouse cells using somatic cell genetic techniques. We shall determine if nucleated cell fragments (karyoplasts) from cells pretreated with interferon can produce the antiviral state in cells and enucleated cytoplasms (cytoplasts) from untreated cell populations. We shall conduct these studies using an autoradiographic assay system from the antiviral state. We shall also investigate the possibility of a distinct nuclearly restricted antiviral state by examining the production of viable vaccinia virions in cells (reconstituted) from karyoplasts and cytoplasts of different origins. We shall further investigate the control of the interferon system by examining phenotypic properties of cells resistant to the growth inhibitory activity of interferon and we shall extend these studies to the effects of interferon on cellular protein synthesis using a 2-D gel analysis. We shall also examine control by continuing our studies on the expression of the interferon system in hybrid cells formed from embryonal carcinoma cells which normally possess an inactive interferon system.

71. GENETIC MAPPING AND PURIFICATION OF INTERFERON GENES

Ruddle, F. H., Yale University, Graduate School, Dept. of Biology, *206 Elm, New Haven, Connecticut*, 06520, United States of America

No summary has been provided to the Smithsonian Science Information Exchange.

72. FROM GENE TO PROTEIN - STRUCTURE FUNCTION AND CONTROL IN EUKARYOTIC CELLS

Berger, S. L., Birkenmeier, C., Reber, S. B., Siegal, G. P., Wallace, D. M., Torrence, P. F., Johnson, M. I., Hitchcock, M. J., Friedman, R. M., Zoon, K. C., Liotta, L. A., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Cellular & Molecular Physiology Section, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

The production of human lymphoblastoid interferon messenger RNA synthesis was characterized in Namalva cells after induction with Newcastle disease virus. The level of interferon mRNA increased 50- to 100-fold within 8 hours of induction. The amount remained constant for an additional 6 hours but began to decrease by 17 hours postinduction. Under the latter conditions the entire cellular complement of interferon mRNA was found free in the cytoplasm. In contrast, 50% of the interferon mRNA was polysome-associated earlier, during active synthesis of the antiviral protein. At both times interferon mRNA was fully translatable in oocytes of Xenopus laevis. Since interferon protein is virtually fully induced 11 hours after the initiation of virus treatment, the shut-off of interferon synthesis cannot be attributed to decay of its mRNA. These results show that control of interferon synthesis in Namalva cells is a function of mRNA translatability, not of mRNA stability. The data explain, in part, why superinduction of interferon mRNA has never been observed in these cells. Immune interferon mRNA was isolated from human lymphocytes stimulated with staphylococcal enterotoxin A. The yield is approximately 200-fold lower than the yield of lymphoblastoid interferon mRNA from Namalva cells.

BIBLIOGRAPHIC REFERENCES: Berger, S.L., and Birkenmeier, C.S. Inhibition of intractable nucleases with ribonucleoside-vanadyl complexes: Isolation of messenger RNA from resting lymphocytes. Biochemistry 18: 5143-51, 1979. Berger, S.L., Hitchcock, M.J.M., Zoon, K.C., Birkenmeier, C.S., Friedman, R.M., and Chang, E.H. Characterization of interferon messenger RNA synthesis in Namalva cells. J. Biol. Chem. 255: 2955-29, 1980.

73. INTERFERON-INDUCED PROTEINS IN MOUSE AND HUMAN CELLS

Gupta, S. L., Rubin, B., Holmes, S. L., Sloan Kettering Inst. for Cancer Research, Interferon Evaluation Program, *410 E. 68th St., New York, New York*, 10021, United States of America

The manner in which interferon treatment results in antiviral, anticellular, antitumor, immunomodulatory, and many

other effects is not understood. We plan to identify and characterize the proteins which are induced in various mouse and human cells upon interferon treatment. Cells will be labelled with radioactive amino acids during interferon treatment, and their extracts will be fractionated by various techniques such as ammonium sulfate fractionation, ion-exchange chromatography, gel filtration, chromatography on double-stranded RNA columns, etc., and various fractions will be analyzed by SDS-polyacrylamide gel electrophoresis in slab gels to identify induced proteins. In such studies, we have already detected certain proteins that are induced in mouse cells (Ehrlich ascites, L929) and in human cells (FS4, GM258) upon treatment with homologous interferon. We suspect that these proteins may be the products of the cellular genetic information that is required to be induced for the development of the antiviral effect of interferon. We plan to investigate the association of these induced proteins with the biological effects of interferon using the antiviral effect as a criterion, and to examine their possible correlation with the known interferon-induced enzymes (a protein kinase and 2'-5' oligoadenylate synthetase) and the kinetics of their induction during interferon treatment and de-induction upon withdrawal of interferon. We also plan to raise antibodies against several of these induced proteins for future studies on the induction process.

74. INTERFERON, DOUBLE-STRANDED RNA AND PROTEIN KINASES

Lengyel, P., Farrell, P. J., Sen, G., Wiegand, R., Ratner, L., Dubois, M. F., Yale University, School of Medicine, Dept. of Molecular Biophysics & Biochemistry, *333 Cedar St., New Haven, Connecticut,* 06510, United States of America

Interferons are glycoproteins whose formation is induced in various animal cells upon infection by any of a large variety of viruses. They are released from the producing cells and attached to other cells, to impair in these the replication of a broad range of viruses. We plan to study the molecular basis of this impairment. Specifically, we want to examine the role, if any, of protein kinase(s) and of endonuclease(s) which are more active in extracts of interferon-treated (mouse or human) cells than in those untreated cells. Both of these enzymes require double-stranded RNA as well as ATP for their action. This requirement is intriguing since double-stranded RNA, a potent inducer of interferon, is formed in the course of the replication of various viruses and, thus, might turn out to be a mediator in the induction of interferon by viruses. To test the relevance of the above findings (obtained in studies with cell extracts) we propose to check if the same proteins whose phosphorylation is promoted by the protein kinase in extracts from interferon-treated cells in the presence of double-stranded RNA are also phosphorylated in intact interferon-treated cells and if this depends on virus infection. Furthermore, we plan to fractionate cell extracts to clarify the steps in the activation of the endonuclease by double-stranded RNA and ATP and the possible role of protein kinase(s) in this process.

75. INTERFERON EVALUATION PROGRAM

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This is part of a broader project. A summary of this subproject is not available.

76. INTERNATIONAL WORKSHOP ON INTERFERON

Krim, M., Memorial Sloan Kettering Cancer Center, *1275 York Ave., New York, New York,* 10021, United States of America

No summary has been provided to the Smithsonian Science Information Exchange.

F. INTERFERON INDUCTION

77. INTERFERON SYSTEM - ACTION, INDUCTION AND REGULATION

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The summary for this project is under entry no. 23.

78. CONTROL OF PROTEIN SYNTHESIS BY DOUBLE-STRANDED RNA

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The biological significance of double-stranded RNA (dsRNA) is reflected in the increasing evidence which indicates that dsRNA exerts a regulatory effect on a broad spectrum of biological systems. Specific effects include (a) the induction of interferon, (b) cytotoxic effects on cells, (c) suppression of tumor development, (d) enhancement of the immune response, and (e) inhibition of protein synthesis. Several striking features common to these physiological mechanisms are (1) the lack of specificity of the dsRNA inducer, (2) sensitivity of physiological levels of dsRNA, and (3) the capacity of viral replicative intermediates to replace pure dsRNA. The broad objective of our research effort has been to determine the molecular basis for these effects, with particular emphasis on the control of protein synthesis by dsRNA, an ubiquitous feature of those biological systems. The reticulocyte lysate is particularly responsive to dsRNA and has provided us with a laboratory model for the study of several biological mechanisms induced by dsRNA similar to those observed in interferon-sensitized cells, including (1) the activation of a protein kinase which regulates protein synthesis, and (2) the activation of a (2'-5') oligo A synthetase whose product promotes nuclease activity. A primary conclusion of our studies - and others' as well - is the importance of phosphorylation-dephosphorylation events in the regulation of translation and transcription. Projected research efforts based on these findings will focus on two areas: (1) Studies on the control of translation will deal with the nature and function of the dsRNA-induced protein kinase in interferon-treated cells including HeLa, human diploid fibroblasts, Krebs ascites, as well as induced Friend erythroleukemia cells. (2) A study on the control of transcription by phosphorylation will be initiated using isolated nuclei of induced and uninduced Friend cells.

BIBLIOGRAPHIC REFERENCES: Daniel Levin, Vivian Ernst, and Irving M. London. Effects of the catalytic subunit of cAMP-dependent protein kinase (Type II) from reticulocytes and bovine heart muscle on protein phosphorylation and protein synthesis in reticulocyte lysates. Journal of Biological Chemistry (1979) 252, 7935-7941. V. Ernst and D. Levin. In situ phosphorylation of eIF-2 in reticulocyte lysates. Fed. Proc. 38:2913, 1979.

79. HOST CELL INTERACTION OF ANIMAL VIRUSES

Marcus, P. I., Whelan, S., Sekellick, M. J., Fuller, F. J., Guidon, P., University of Connecticut, Storrs Campus, School of Liberal Arts & Sciences, Dept. of Biological Science, *Storrs, Connecticut,* 06268, United States of America

OBJECTIVES: Interferon induction: (1) to define the mechanism of interferon induction using as a model a system in which a single (plus or minus) RNA D1-011 particle of vesicular stomatitis virus per cell induces a quantum yield of interferon and two or more particles per cell produce little or no interferon; (2) to define the molecular nature of the inducer moiety of Sindbis virus, its ts-mutants and early passage D1 particles; and (3) to define the mechanism that regulates which of two types of interferon dose (multiplicity of inducer)-response (interferon yield) curves is expressed during induction. Interferon action: to define further the inhibition of primary transcription by interferon action in some cell types infected with certain negative-strand viruses. Cell killing by viruses: to define the viral genes required for cell-killing by different viruses and to determine the nature and mode of action of the putative cell killing factor(s). Defective-interfering particle interference: (1) to obtain clonal populations of D1 particles through single-cell isolation procedures, and (2) to determine the role of D1 particles in persistent infection, with an emphasis on 'cell persistence' through interferon action and cell sparing. Intrinsic interference: to determine the mechanism of intrinsic interference.

BIBLIOGRAPHIC REFERENCES: Interferon Induction by Viruses. III. Vesicular Stomatitis Virus: Interferon-inducing particle activity requires partial transcription of gene N. Philip I. Marcus and Margaret J. Sekellick. J. General Virology 47:89-96, 1980. Interferon Induction by Viruses. IV. Sindbis Virus:

Early Passage Defective-interfering Particles Induce Interferon. Frederick J. Fuller and Philip I. Marcus. J. General Virology, in press, 1980.

80. BIOLOGICAL ACTIVITY OF AGENTS ENTRAPPED IN LIPOSOMES

Magee, W. E., University of Texas, San Antonio Campus, School of Science & Mathematics, Division of Allied Health & Life Sciences, *San Antonio, Texas*, 78285, United States of America

Liposomes (phospholipid vesicles) are being used to deliver selected materials to cells in vitro and in the animal. The vesicles are prepared from various phospholipids and are target-directed to cells of interest by attachment of cell-specific antibodies, antibody fragments or other ligands to the exterior of the particles. Optimal conditions for liposome-cell interaction are being determined for transfer of intact nucleic acids such as infectious viral RNA and poly(I): poly(C) to the cytoplasm of target cells.

Present studies on the use of liposomes for delivery of inducers of interferon in the animal are being expanded to include a study of the responding cells and their interactions. Target-directed liposomes are used to deliver chemotherapeutic agents which inhibit tumor growth and prevent or reverse virus-induced leukemia. These studies are expected to expand our knowledge of how liposomes interact with target cells and how liposomes may be utilized to deliver nucleic acids, drugs, and other agents to selected target tissues.

81. THE INDUCTION OF HUMAN INTERFERON IN C-10 CELLS

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No summary has been provided to the Smithsonian Science Information Exchange.

G. INTERFERON PRODUCTION

82. LEUKOCYTE INTERFERON PRODUCTION

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The purpose of this research development program is to continue to improve conditions for the production and purification of human leukocyte interferon. The present incubation system, utilizing Newcastle disease virus (NDV) as inducer, bovine milk solids as protein support and human mixed peripheral blood leukocytes for production, will be optimized and the scale of production expanded.

Alternate systems will also be developed, with special emphasis on replacing the bovine material with purified, characterized fractions of human plasma. It is hoped that the results of these studies will be the increased availability of human leukocyte interferon acceptable for clinical use.

83. THE PRODUCTION, PURIFICATION AND CHARACTERIZATION OF HUMAN LEUKOCYTE INTERFERON FOR CLINICAL TRIALS (ABBREV)

Krim, M., Memorial Sloan Kettering Cancer Center, *1275 York Ave., New York, New York*, 10021, United States of America

A human leukocyte interferon (HuIFN-alpha) production laboratory has been developed collaboratively between the Theodor Kocher Institute of the University of Berne, Switzerland and the Sloan-Kettering Institute, New York. With minor modifications, methods developed by Dr. Kari Cantell in Finland are being followed. In 1979, the average yield of crude interferon per WBC concentrate ('buffy coat') has been 1.9 x 10 to the 6th power IU and the average recovery of activity following purification has been 41%. As both partially purified interferon yields per 'buffy coat', and the number of 'buffy coats' the laboratory could process each day progressively increased, the effort had the potential of becoming a source of significant amounts of HuIFN-alpha suitable for clinical trials. This grant has supported a 3-month period of research and development in improved production and purification, as well as a significant scale up of operations, which have provided HuIFN-alpha of titer and specific activity similar to Finnish material. It is presently used in clinical trials at the M.D. Anderson Hospital in Houston and at the Memorial Slon-Kettering Cancer Center in New York.

84. DEVELOP AND DELIVER HUMAN LEUKOCYTE INTERFERON

Beardmore, W., Warner-Lambert Co., Pharmaceutical Research Division, *2800 Plymouth Rd., Ann Arbor, Michigan*, 48106, United States of America

Research, process development, and production of fifty billion units of human leukocyte interferon.

85. EVALUATION OF CROSS-FLOW FILTRATION IN THE PRODUCTION, PURIFICATION AND CONCENTRATION OF HUMAN LEUKOCYTE INTERFERON (TYPE I)

Friedman, L. I., American Red Cross, Blood Services Laboratories, *9312 Old Georgetown Rd., Bethesda, Maryland*, 20014, United States of America

No summary has been provided to the Smithsonian Science Information Exchange.

86. DEVELOPMENT OF MAMMALIAN CELL MICROCARRIER CULTURING SYSTEMS FOR THE PRODUCTION OF BIOLOGICAL PRODUCTS FOR HUMAN USE

Mizrahi, A., Israel Inst. for Biological Research, Dept. of Biotechnology, *P.O. Box 19, Ness Ziona, Israel*

OBJECTIVE: Research and development (R and D) of microcarrier culturing systems for the production of biological products for human use, such as interferon for treatment of cancer patients.

APPROACH: R and D of new available and planned to be synthesized beads. These beads (microcarriers) are substrates for anchorage-dependent cells (ADC). Human fibroblast interferon is produced after suitable induction by ADC. R and D will be carried out to investigate physicochemical, as well as biological, factors in the growth of the ADC in the newly investigated microcarriers.

PROGRESS: A new project, only in the initial stages of R and D.

87. REGULATION OF GENE EXPRESSION IN HUMAN CELLS

Sehgal, P. B., Rockefeller University, Virology Lab., *66th & York Ave., New York, New York*, 10021, United States of America

The regulation of gene expression in mammalian cells is complex. Human fibroblast interferon production in poly(I).poly(C)-induced cultures of diploid human fibroblasts (FS-4 strain) is subject to a post-transcriptional (translational) repressor mechanism which appears to inactivate or degrade interferon mRNA. An inhibition of this repression by appropriate use of inhibitors of RNA (e.g. DRB, 5,6-dichloro-1-beta-D-ribofuranosyl-benzimidazole) and protein (e.g., cycloheximide) synthesis leads to a marked (50-fold) paradoxical enhancement of human interferon yields ('superinduction'). Increased interferon mRNA stability in superinduced cells is the major contributor to this phenomenon. We intend to: 1) Develop a cell-free system to investigate the regulation of human fibroblast interferon mRNA stability. This line of work should lead to the eventual biochemical characterization of the molecules involved in the post-transcriptional repressor mechanism. 2) Carry out further investigations of the synthesis, structure and function of human fibroblast interferon mRNA. We will investigate the size of the fibroblast interferon transcription unit, the regulation of transcription, and the role of 3'-terminal poly(A) in the appearance of cytoplasmic interferon mRNA and in its function, and determine the structure of the 5' end of interferon mRNA. 3) Develop improved procedures to enhance human fibroblast interferon yields from diploid cell cultures and evaluate the use of DRB as a superinducing adjuvant in vivo.

These investigations not only address fundamental questions in molecular biology but also should provide a sound basis for obtaining larger amounts of human fibroblast interferon that may be used in the clinic against a variety of viral and neoplastic diseases of man.

Investigations of the mechanism of action of DRB, an inhibitor of hnRNA and mRNA synthesis and also of the synthesis of adeno-, papova-, and oncornavirus RNA in mammalian

and avian cells, represent a study of transcriptional regulation in human cells. DRB blocks RNA synthesis at multiple discrete sites within a few hundred nucleotides of the promoter in several of AD2-specific transcription units in HeLa cells. Furthermore, these sites appear to function even in the absence of DRB and lead to premature RNA chain termination. DRB is thus a probe for transcriptional attenuation. (Text Abridged.)

88. RESEARCH AND DEVELOPMENT OF HUMAN LYMPHOBLASTOID INTERFERON

Mizrahi, A., Traub, A., Israel Inst. for Biological Research, Dept. of Biotechnology & Biochemistry, *P.O. Box 19, Ness Ziona, Israel*

OBJECTIVE: Research and development (R and D) of methods for large scale production of human lymphoblastoid interferon for use in cancer patients.

APPROACH: Cells from the Namalva human lymphoblastoid cell line, which is grown in suspension, produce lymphoblastoid interferon after viral induction.

The R and D is carried out using laboratory bench and pilot plant scale fermentors. Cell cultures are semicontinuous cultures, while the interferon induction is carried out in batch cultures. R and D is carried out to improve the growth medium, and to investigate several physicochemical factors in the interferon production process. R and D of the purification and concentration of the lymphoblastoid interferon is planned.

PROGRESS: 10 to the 5th power units of crude lymphoblastoid interferon per 10 to the 7th power cells per ml culture are harvested from the up-to-date developed production process.

89. ECONOMIC PRODUCTION OF THE ANTIVIRAL/ ANTICANCER AGENT HUMAN INTERFERON

Christie, B. F., McInnes, J., Harris, R., South Australian Inst. of Technology, School of Pharmacy, *North Terr., Adelaide, S. Australia 5000, Australia*

OBJECTIVE: To economically mass-produce human interferon in bacteria.

APPROACH: The approach entails 1) production and partial purification of human fibroblast and lymphoblastoid interferon messenger ribonucleic acids (mRNA), 2) preparation of complementary DNA (cDNA), 3) cloning of cDNA using PBR 322 in E. coli, 4) direct selection of interferon producing clones.

PROGRESS: 1. Whole cell poly A mRNA containing fibroblast mRNA has been produced on a mg scale and purified 50-fold. 2. Production of lymphoblastoid interferon mRNA from measles infected Namalva cell line is under way.

90. HUMAN IMMUNE INTERFERON PREPARATION ANTITUMOR EFFECT

Johnson, H. M., Georgiades, J. A., Blalock, E. J., Patten, E. D., University of Texas, Medical Branch at Galveston, School of Medicine, Dept. of Microbiology, *300 University Blvd., Galveston, Texas,* 77550, United States of America

We propose to develop a system for large-scale production of human immune (type II) interferon for possible future application in clinical studies for therapy of cancer and in mechanistic studies. Further, we plan to make the system as efficient as that used for large-scale production and purification of human leukocyte interferon, which is currently being used in clinical trials. Our preliminary data suggest that our objective is reasonable. Using staphylococcal enterotoxin A as inducer, we estimate that we can produce 1.25 x 10 to the 6th power NIH reference units of immune interferon from 500 ml of blood. This is comparable to the yields reported for human leukocyte interferon. We can purify this interferon 5,000-fold to specific activity greater than 10 to the 6th power units/mg protein using a combination of adsorption, hydrophobic, and gel filtration chromatography with approximately 80% yield of starting interferon. In addition to production and purification studies, the mechanism of the antitumor (anticellular) effects of immune interferon will be studied. We will determine the in vitro antitumor (anticellular) effects of the purified immune interferon in a colony system with transformed human cells. Immune interferon treated cells will transfer viral resistance to untreated cells. The transfer phenonemon will be tested for in the anticellular effects of immune interferon. This type of cell-cell interaction may play an important role in the antitumor effects of interferon. Preliminary data in our laboratory suggest potent antitumor effects. Recent studies have indicated in the mouse system that immune interferon preparations are 100 times more effective on a dose basis in preventing and controlling osteogenic sarcoma than virus-induced interferon. These animal studies suggest that human immune interferon may prove a much more effective antitumor agent than human leukocyte interferon. It is important, therefore, that the appropriate methodologies be available for large-scale production and purification and that mechanistic studies be done on the anticellular effect. Safety of the produced interferon will be determined in animal studies.

91. EQUIPMENT FOR LARGE-SCALE PRODUCTION OF HUMAN IMMUNE INTERFERON

Johnson, H. M., University of Texas, Medical Branch at Galveston, Graduate School of Biomedical Sciences, Dept. of Microbiology, *Galveston, Texas,* 77550, United States of America

No summary has been provided to the Smithsonian Science Information Exchange.

92. HUMAN INTERFERON PRODUCTION FOR ANTITUMOR STUDIES

Tan, Y., Inoue, M., Okamura, H., University of Calgary, Medical Biochemistry Division, *26th St. & 26th Ave. N.W., Calgary, Alberta,* T2N 1N4, Canada

Human inteferon from C-10 cells was purified with good recovery by an immunisorbent column. The pure interferon was characterised for its amino acid composition and N-terminal amino acid sequence. The interferon was also chemically and enzymatically cleaved to provide several fragments (MW 18,000; 15,000; 14,000; 12,000 and 6,000).

BIBLIOGRAPHIC REFERENCES: McPherson, T.A., Tan, Y.-H. (1980), J.N.C.I., Phase I Study of human fibroblast interferon in human malignancy, in press. Tan, Y.-H. (1980). Methods of Enzymology Induction and Production of Human Interferon from continuous lines of modified fibroblasts, in press.

93. MOLECULAR CLONING AND EXPRESSION OF SV40 SMALL-T AND LARGE-T ANTIGENS FOR EXPRESSION BACTERIA - MODEL FOR PRODUCTION OF HUMAN INTERFERON

Jay, E., University of New Brunswick, Dept. of Chemistry, *P.O. Box 4400, Fredericton, New Brunswick,* E3B 5A3, Canada

OBJECTIVES: 1. To clone the SV40 small-t antigen gene isolated from SV40 DNA into a plasmid vector (pBR322) for transformation of E. coli x1776 and expression of SV40 small-t antigen. 2. To clone the SV40 large-T antigen gene obtained by reverse transcription of the processed poly A containing mRNA for large-T into a plasmid vector (pBR322) for transformation of E. coli x1776 and expression of SV40 large-T antigen. 3. Using the above as model, to clone a human interferon gene into the plasmid vector (pBR322) for transformation of E. coli x1776 and expression of human interferon.

APPROACH: Many attempts to clone a eukaryotic gene into a cloning vector for specific expression of the eukaryotic protein have not met with success thus far. We believe that the most likely explanation for this is that the eukaryotic mRNAs lack the appropriate initiation signals for recognition by prokaryotic ribosomes. We propose to chemically synthesize an E. coli ribosome binding (initiation) site, and attach it in front of the eukaryotic SV40, t, T and human interferon genes prior to their insertion into the cloning vector. This we hope will assure their expression in bacteria.

94. IMMUNE INTERFERONS - PRODUCTION AND ROLE IN IMMUNITY

Kamin, R. M., Epstein, L. B., Hirabayshi, J., University of California, San Francisco Campus, School of Medicine, *551 Parnassus Ave., San Francisco, California,* 94122, United States of America

Polyclonal activation or specific antigenic stimulation of lymphocytes initiates a complex series of events, including the elaboration of the anti-viral protein, immune interferon. This mediator is known to be a product of thymus-derived lymphocytes (T cells) of mouse or human origin. The report on interferon detected in supernatants of cultures comprised of highly enriched proportions of human non-thymus derived lymphocytes (B cells) suggests that activated murine B cells might also elaborate interferon. The present proposal will investigate the

production of immune interferon by murine B cell subsets in response to stimulation with mitogens or antigens. Additionally, the murine T cell subset responsible for interferon production will be characterized, and the extent and genetic requirements of macrophage participation will be studied. Murine plasmacytomas and thymomas will be examined as possible abundant sources of T cell- or B cell-derived immune interferon preparations. Attempts will be made to partially purify the interferon from such preparations. Both crude and such partially purified interferon preparations will be tested for their effects on the immune response in assays involving both the in vitro proliferative response to mitogen or antigen stimulation and in vitro antibody response. The cell targets of the interferon immunoregulatory activity in the immune response will be characterized by pretreating isolated macrophage T cell or B cell populations with interferon prior to recombining them in cultures for an immune response and by performing comparative limiting dilution analysis of cells cultured in the presence and absence of interferon.

95. ANTILEUKEMIC FACTORS AND INTERFERONS PRODUCED BY DIFFERENTIATING MONOLAYER CULTURES OF HUMAN BONE MARROW STROMAL CELLS

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No summary has been provided to the Smithsonian Science Information Exchange.

96. IMMUNE INTERFERON PRODUCTION IN MOUSE SPLEEN CELL CULTURES

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OBJECTIVE: It has been demonstrated that the interferons can inhibit proliferation of many tumor cells in addition to arousing the immune system for tumor destruction. This is especially true for immune interferon.

At present, it is not totally clear if the T lymphocyte is the sole cell source of immune interferon. The objective of this project is to determine if in the mouse system, the T cell is the ultimate source of immune interferon or if other cells (B lymphocytes, macrophages) can also produce immune interferon. If cells other than T lymphocytes can be induced to make immune interferon, we will try to determine the nature of any cooperative mechanism(s) among these cells.

APPROACH: We will separate mouse spleen cells using conventional means to produce T and B lymphocyte and macrophage enriched populations. By stimulating whole spleen cell suspensions or fractions enriched for each of the described cells with concanavalin A, concanavalin A covalently bound to Sepharose beads, or with galactose oxidase, we will assay for immune interferon production. Finally, we wish to determine if any of these cells (notably the T lymphocyte) can be induced to secrete a cytokine other than interferon that in turn is capable of stimulating B cells, macrophages or perhaps other T cells to produce immune interferon.

II. DEVELOPMENT AND STUDIES OF OTHER ANTICANCER AND ANTIVIRAL AGENTS

A. CLINICAL STUDIES OF ANTICANCER AND ANTIVIRAL AGENTS

97. PHARMACOLOGY AND ANTIVIRAL EFFECT OF DS-POLYNUCLEOTIDES

Freeman, A. I., Roswell Park Memorial Inst., *666 Elm St., Buffalo, New York*, 14263, United States of America

Varicella zoster (V-Z) infections in patients with cancer are common and may be severe. Both adenosine arabinoside and preformed human interferon (IF) have demonstrated efficacy in established herpes infections.

Currently, we are running a randomized, double-blind study in cancer patients with herpes zoster, where half the patients receive polyinosinic polycytidylic acid (Poly I:C) and the other half receive a placebo to determine whether Poly I:C can alleviate herpes zoster. To date, 40 patients have been entered on the study and 38 are evaluable. The Poly I:C treated patients had shorter times to (1) shut-off of new vesicles, (2) pustulation, and (3) crustation, but the differences were modest and the number in each too small for statistical evaluation. We also showed that patients with markedly depressed T-cells (500) or with grossly deficient cellular response to V-Z antigen did worse; and that patients in relapse had significantly lower IF levels both in serum and in vesicle fluid and lower proliferative activity of lymphocytes to V-Z antigen when compared to patients in remission of their cancer. We have also treated three children exposed to varicella with Poly I:C during the incubation period. None of these three children developed clinical varicella.

We now propose (1) to complete the clinical randomized double-blind study in herpes zoster and (2) to study Poly I:C/Poly-L-lysine in (a) children with cancer who have been exposed to varicella and in (b) cancer patients with documented viral pneumonia. Poly I:C-LC results in much higher levels of serum IF compared to unmodified Poly I:C. (3) We also propose to develop a thiolated analog of Poly I:C for human testing which appears to avoid the hyporesponsive state following Poly I:C and thus allows the use of multiple doses of thiolated Poly I:C during a viral illness.

BIBLIOGRAPHIC REFERENCES: Kende, G., Wajsman, Z., Thomas, P.R.M., Freeman, A.I.: Chronic hematuria and localized bladder damage following combined cyclophosphamide and local radiotherapy. J. Surg. Oncol. 12:169-174, 1979. Kedar, A., Khan, A.B., Mattern, J.Q.A., Fisher, J., Thomas, P.R.M., and Freeman, A.I.: Autoimmune disorders complicating adolescent Hodgkin's disease. Cancer 44(1):112-116, 1979.

98. ACUTE LEUKEMIA COOPERATIVE STUDY GROUP B

Haurani, F. I., Thomas Jefferson University, Jefferson Medical College, Dept. of Medicine, *1025 Walnut St., Philadelphia, Pennsylvania*, 19107, United States of America

The objective of this study is to participate in the studies of Cancer and Leukemia Group B. The aim of this group is (1) to screen new chemotherapeutic agents in patients with cancer (Phase I study), (2) to explore the clinical pharmacology of known effective drugs (Phase II studies). This consists of optimal dose, frequency of administration, sequence of administration, combination of agents and route of administration. Again, emphasis will be on the importance of consolidation and reinduction in the duration of remission and survival.

Besides chemotherapeutic agents, interdisciplinary programs have been developed to include radiotherapy, immunotherapy, anti-viral chemotherapy, surgery and psychiatry. Ancillary treatment which includes platelet transfusion, granulocyte transfusion and sterile environment and antibiotics will be evaluated.

We have, presently, the following centers working with us: (1) Wilmington Medical Center, (2) Albert Einstein Hospital/Northern Division, (3) Albert Einstein Hospital/Daroff Division, (4) Cooper General Hospital, (5) John F. Kennedy Hospital, (6) Lankenau Hospital, (7) Medical College of Penn, (8) Philadelphia Naval, (9) Portsmouth Naval, (10) Our Lady of Lourdes Hospital, (11) Woodbury Memorial Hospital.

BIBLIOGRAPHIC REFERENCES: Haurani, F.I., and Masse, A.T.: Serine hydroxymethyltransferase and the stimulated human lymphocyte. J. of the R.E.S. 21:231-235, 1977. Maurani, F.I.: Folate analogues and thymidylate synthetase of the PHA stimulated human lymphocyte. J. of the R.E.S., Dec. 1977.

99. DEVELOPMENT OF A CENTRAL FACILITY TO STUDY ADENINE ARABINOSIDE

Whitley, R. J., University of Alabama in Birmingham, School of Medicine, Dept. of Pediatrics, *1919 7th Ave. S., Birmingham, Alabama*, 35233, United States of America

The overall objectives of this contract remain unchanged; however, a new protocol for the study of herpes zoster is being submitted. This protocol will extend the observations and data

derived from the blind cross-over study of zoster. Thus, patients will be randomized into therapy and placebo groups early (within 48 hours of appearance of vesicles) in the course of illness. The purpose of this protocol is to answer the questions of potential efficacy in preventing cutaneous or visceral dissemination and the question of potential additive toxicity in patients already receiving immunosuppressive agents.

B. DEVELOPMENT AND STUDIES OF NUCLEIC ACID AND BASE ANALOGS

100. CHEMOTHERAPY OF CANCER AND HERPES-ASSOCIATED DISEASE

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The overall objective of this proposal is to develop and improve the chemotherapy of cancer and herpesvirus-associated diseases by taking advantage of some of the unique properties of thymidine kinase, DNase and DNA polymerase. This approach should also provide potential new leads to the diagnosis and the study of the etiology of herpes-related diseases.

To achieve this objective, experiments planned for this period are designed to enable the following: (a) Further studies of the alternative substrates of herpes simplex virus type I, herpes simplex virus type II and varicella-zoster virus specific thymidine kinase. (b) Development of thymidine or deoxyuridine analogs which could act as the substrate or inhibitor of human thymidine kinases, but not as the substrate of human thymidine phosphorylase. (c) Studies of the herpes virus induced DNase. (d) Studies of substrate and inhibitor specificities of purified herpes virus induced DNA polymerase. (e) Purification of antibodies against herpes virus specific DNase and thymidine kinase in order to elucidate the association of human tumors with herpes viruses.

101. ANTIHERPESVIRAL ACTIVITY OF ARA-T

Gentry, G. A., University of Mississippi, Medical Center, School of Medicine, Dept. of Microbiology, *2500 N. State St., Jackson, Mississippi*, 39216, United States of America

Our overall objective is to develop chemotherapeutic tools for the treatment of herpes simplex. We will continue our study of arabinosylthymine (araT) and related compounds that act in a similar manner (i.e., phosphorylated by herpesviral deoxythymidine kinase (dTK) but not by mammalian cytosol dTKs). We will prepare radioactive araT in order to study its intracellular metabolism as well as to explore the development of a rapid assay for herpes simplex virus (HSV) in clinical specimens by measurement of its incorporation into DNA.

We will also compare dTKs from several sources with herpesviral dTKs and further characterize the features that tend to differentiate the viral dTKs as a group from the mammalian dTKs. Finally we will compare an araT donor, 5-methyl araC, with araT and with other antiherpesviral compounds for its ability to protect hamsters against equine herpesvirus type 1 (EHV-1), and we will extend this comparison to include immunosuppressive and teratologic potential.

102. ANTIVIRAL ACTIVITY OF SOME OLIGO 5-IODO-2'-DEOXY-URIDINES

Ho, B., Levy, C. C., Cheng, Y., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Laboratory of Molecular Biology, *Baltimore, Maryland*, 21201, United States of America

Oligonucleotides of 5'-iodo-2'-deoxy-uridine are synthesized by the triester method, i.e., all the intermediates plus starting nucleotide functional groups, such as 5', 3' hydroxyl groups and phosphate groups, were protected. The 5' hydroxyl was protected with trityl groups and 3' hydroxyl by benzoyl groups. The phosphate was protected with rho-Cl-phenol and beta-cyanoethanol. Removal of protecting group was achieved by the treatment of 2% benzene sulfonyl tetrazole followed with the treatment of concentrated ammonia at 50 degrees C. The effectiveness of oligo nucleotides thus synthesized is being tested against herpes simplex virus.

103. IODODEOXYURIDINE, IODO-DNA, AND BIOLOGICAL ACTIVITY

Prusoff, W. H., Yale University, School of Medicine, Dept. of Pharmacology, *333 Cedar St., New Haven, Connecticut*, 06510, United States of America

This grant has enabled the development of a unique antiviral agent, 5-iodo-5'-amino-2',5'-dideoxyuridine (AIU; AIdUrd) which, because it is activated only by a viral encoded enzyme, has good antiviral activity in vitro and in vivo with no host toxicity.

The objectives are to synthesize additional novel antiviral and anticancer nucleoside analogues, to evaluate their spectra of biological activity, and to determine the site of inhibition and mechanism of action of those compounds which have good inhibitory activity. Both antiviral and anticancer activities are evaluated initially in cell culture, and then in animal systems using a variety of virus infections as well as a number of transplanted neoplasms.

BIBLIOGRAPHIC REFERENCES: Lin, T.-S., and Prusoff, W.H., A novel synthesis and biological activity of several 5-hale-5'-amino analogs of deoxyribopyrimidine nucleosides. J. Med. Chem., 21, 106-109, 1978. Lin, T.-S., and Prusoff, W.H., Synthesis and antiviral activity of 5-methylamino-2'-deoxycytidine. J. Carbohydrates, Nucleosides, Nucleotides, 5, 15-22, 1978.

104. PYRIMIDINES FOR ANTICANCER AND ANTIVIRAL CHEMOTHERAPY

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Our prior studies of carbocyclic analogs of purine nucleosides and of a few pyrimidine nucleosides have uncovered significant anticancer and antiviral activity among compounds with this structure, in which the furanose ring of nucleosides is replaced by a cyclopentane ring. The observed activities by certain carbocyclic analogs, the importance of pyrimidine nucleosides among clinically active anticancer drugs, and the preeminence of nucleosides among clinically useful antiviral agents indicate a potentially important role for carbocyclic pyrimidine nuleoside analogs. For these reasons, this proposed, collaborative research program includes the synthesis of new carbocyclic pyrimidine nucleoside analogs that are either congeners of the active carbocyclic analogs or are analogs of known active pyrimidine nucleosides. The new analogs are being evaluated in vitro for anticancer activity and for antiviral activity. Analogs that show significant activity in vitro are to be further evaluated in vivo. Biochemical studies are designed to determine if active analogs selectively inhibit virus-induced enzymes.

105. BIOCHEMISTRY OF NEW ANTIVIRAL AND ANTICANCER AGENTS

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Biochemical parameters, involved in antiviral and antitumor activity of pyrimidine purine nucleosides, newly synthesized and to be synthesized in the laboratories of the Sloan-Kettering Institute, will be investigated. These parameters include deamination, phosphorylation, tissue distribution and metabolism in vitro and in vivo.

Structural requirements of these and related compounds for deamination and/or phosphorylation with human and rodent normal and malignant tissue homogenates and virally induced enzyme preparations will be established, with the ultimate goal of obtaining insight into selectivity and specificity of these compounds as antiviral and antitumor agents.

Data on selected purified kinases carried or induced by viruses or present in tumor cells will be compared functionally and structurally with reported data on kinases derived from a variety of normal and malignant tissues.

106. SYNTHESIS AND EVALUATION OF NEW PURINE-LIKE NUCLEOSIDES AS ANTITUMOR AND ANTIVIRAL AGENTS

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No summary has been provided to the Smithsonian Science Information Exchange.

107. PHOTOSYNTHESIS OF ANTIVIRAL AND ANTITUMOR DRUGS

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The synthesis of novel nucleosides by the photochemical cyclization of the adducts of enaminonitriles and ribose. These nucleosides are being tested as antitumor agents.

108. POLYRIBONUCLEOTIDES AS TUMOR AND VIRUS INHIBITORS

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The proposed study is based upon our finding that certain polyribonucleotides have much greater potency as antitumor agents than the antitumor purines from which they are derived. An additional set of polyribonucleotides, prepared by enzymatic polymerization, will be used to test the hypothesis that these compounds are acting as polymeric drugs or drug delivery systems. A study of immobilization of various synthetic and degradative enzymes will be undertaken. These enzymes will be used in the synthesis and characterization of a carefully defined set of homopolydeoxyribonucleotides which will be evaluated as antitumor and antiviral agents. A comparison of the physicochemical properties of the DNA and RNA analog polymers will be made to reveal subtle differences in intra- and interchain interactions arising from conformational differences in the polymers.

BIBLIOGRAPHIC REFERENCES: W.M. Scovell, V. Amarnath, and A.D. Broom, Nucleic Acids Research, 6, 1049(1979) Raman Spectral Studies of Poly(1-methylinosinic acid). A.D. Broom, V. Amarnath, R. Vince, and J. Brownell, Biochim. Biophys. Acta, 563, 508 (1979).

109. NUCLEOSIDE, NUCLEOTIDE AND POLYNUCLEOTIDE ANALOGS

Bergstrom, D. E., University of California, Davis Campus, School of Letters & Science, Dept. of Chemistry, *Davis, California*, 95616, United States of America

The reaction of pyrimidine and pyrrolo(2,3-d) pyrimidine nucleoside derived organopalladium intermediates with olefins will be examined. The resulting nucleoside analogs are of interest for enzyme studies and as potentially biologically active compounds. Investigation at the nucleoside level will be concentrated on the coupling of allylic chlorides and fluoroolefins to the C-5 position of 2'-deoxyuridine. Derivatives of 2'-deoxyuridine substituted at C-5 by fluorinated two and three carbon side chains will be tested for antiviral activity, cytotoxicity and as an antagonist for enzymes in the biosynthetic pathway to 2'-deoxythymidine 5'-monophosphate.

The reaction of mercurated poly(U) and poly(C) with lithium palladium chloride and allylic chlorides will be examined in detail. The synthesis of polynucleotides substituted at C-5 by aliphatic side chains terminating in the amino group is our primary synthetic target. Once achieved, methods for transforming the amino function to useful affinity labels and reporter groups will be investigated. Reactions which can be applied with a high degree of selectivity at the polynucleotide level will be studied.

A series of tubercidin 5'-triphosphate and 3', 5'-monophosphate derivatives substituted at C-5 will be prepared in order to probe the active-site specificity of enzymes utilizing ADP, ATP, cAMP-dependent protein kinase, pyruvate kinase, phosphoglycerate kinase, hexokinase and phosphofructokinase. New synthetic routes to modified nucleosides structurally resembling coformycin will be investigated. Routes for expansion of the heterocyclic rings of 2'-deoxyuridine and inosine are to be examined.

110. SYNTHESIS AND STUDY OF ANTITUMOR AGENTS

Bardos, T. J., Ho, Y., State University of New York, Health Science Center at Buffalo, School of Pharmacy, Dept. of Medicinal Chemistry & Biochemical Pharmacology, *3435 Main St., Buffalo, New York*, 14214, United States of America

The biological activities and chemotherapeutic application of partially thiolated polycytidylic acid (MPC) and of its double-helical complex with polyinosinic acid (poly(I) MPC) will be investigated in various cell culture systems and animal experiments. New analogs will be synthesized by partial thiolation of synthetic polynucleotides (copolymers), tRNAs and DNA and studied as antitemplates against a spectrum of DNA- and RNA-directed polymerases, in order to determine the structure-activity relationships and selectivities of this new class of macromolecular inhibitors and to develop their potential as novel antineoplastic and antiviral agents.

111. BINDING OF DRUGS TO NUCLEIC ACIDS

Tsai, C., Reinhardt, C. G., Carpenter, R. E., Kent State University, School of Arts & Sciences, Dept. of Chemistry, *Hilltop Dr., Kent, Ohio*, 44240, United States of America

The goal of this research is to elucidate the stereochemistry of drug-nucleic acid interactions and to understand the mechanisms of drug action of a wide spectrum of drugs and antibiotics which exert their pharmacological action by binding to nucleic acids (DNA and RNA). This research proposal describes efforts in applying the physico-chemical techniques such as molecular co-crystallization, X-ray diffraction, and spectroscopic techniques to the studies of the interaction of nucleic acids with anticancer drugs and antiviral agents (such as antiviral drugs and interferon inducers tilorone and its derivatives). The results of these studies may provide useful information for the rational design of more effective anticancer drugs, antiviral agents and interferon inducers. Highlights of current research: Co-crystallization of tilorone and its derivative (DEAP-fluoranthene) with various complementary dinucleoside monophosphates has been successful. Co-crystallization of ethidium bromide with deoxydinucleoside monophosphates has been successful. X-ray crystallographic studies of some of these crystals are in progress.

BIBLIOGRAPHIC REFERENCES: Conformational Flexibility in DNA Structure and Its Implications in Understanding the Organization of DNA in Chromatin, by H.M. Sobell, Chun-che Tsai, S.C. Jain and T.D. Sakore, Phil. Trans. R. Soc. Lond. B. 283, 295 (1978). Stereochemistry of Drug-Nucleic Acid Interactions and Its Biological Implications, by Chun-che Tsai, Annual Reports in Medicinal Chemistry, 13, 316 (1978).

C. DEVELOPMENT AND STUDIES OF OTHER ANTICANCER AND ANTIVIRAL AGENTS

112. NEW NATURAL ANTINEOPLASTIC QUASSINOIDS AND LIMONOIDS

Polonsky, J., Varon, Z., Merrien, A., Varenne, J., Centre National de la Recherche Scientifique, *15 Quai Anatole France, 75700 Paris, France*

The objective is to undertake an extensive search of genera of the Simaroubaceae and the Meliaceae from the French possessions - Guyana and New Caledonia - for new quassinoids and limonoids, respectively. These compounds are important for their antineoplastic activity. Over the last few years some idea of the structure-activity relationship has been established and therefore the semisynthesis - conversion of inactive to active quassinoids - is also included in the project.

My unit at the Institute (at Gif-sur-Yvette) is a leading authority on the chemistry of these structure types; examples of our work are, for instance, our structure elucidations of glucarubinone, similacalactone D and the different C-15 esters of bruceolide (bruceins A, B, C).

The Institute is more than adequately equipped to carry out this study, with the availability of a wide range of instrumentation and a pilot plant laboratory, but in particular because of my large collection of standard samples of quassinoids which is invaluable in hastening the identification procedures.

The bioassays for antineoplastic activity have been (supplier No. 933F) and will continue to be done by Dr. M. Suffness, NIH and Prof. G.R. Pettit, Cancer Research Institute, Tempe, Arizona; the antiviral activity is being tested by Dr. M. Gero (I.C.S.N., Gif-sur-Yvette).

113. DEVELOPMENT OF THERAPEUTIC AGENTS AGAINST CANCER

Dhar, M. M., Central Drug Research Inst., *Lucknow, Uttar Pradesh, India*

SCOPE: The present project, now in its tenth year of operation, provides for the collection and identification of Indian plants, the preparation of extracts according to CCNSC

protocol, the shipment of such extracts to CCNSC for bioassay, the transmittal of screening results to CDRI, the re-collection of active plants and the fractionation of active plants aided by CCNSC bioassays of fractions to yield the active agents. The CDRI synthesizes about 600 new compounds of about 30 variables every year as anti-tumor agents, antifertility agents, antiviral agents, central nervous system agents, polypeptides of biological interest, anthelmintics, etc. The potential antitumour agents have been of special interest to CCNSC and included nucleosides, hetero analogs of podophyllotoxin, ellipticin, and rifamycin. Under the present proposal both the program involving Indian plants and the synthetic chemistry program will be continued and increased.

114. REACTIVE HETEROCYCLES - CANCER AND BIOMECHANISM

Rastetter, W. H., Massachusetts Inst. of Technology, School of Science, Dept. of Chemistry, *77 Massachusetts Ave., Cambridge, Massachusetts*, 02139, United States of America

PART I: The synthesis of reactive epoxides of the arene oxide and oxepin class will be carried out. Nucleophilic additions to these reactive classes of epoxides will be studied in relation to the carcinogenic activation of polycyclic aromatic hydrocarbons, and in relation to the total synthesis of antiviral agents potentially useful in the treatment of virally caused cancer.

PART II: Model systems will be studied to mimic the mechanism of carcinogenic activation of polycyclic aromatic hydrocarbons and the mechanism of similar oxidative transformations by the flavin cofactored monooxygenases.

PART III: Reactive crown ethers, macrocyclic reagents capable of both complexing and reacting selectively with substrates in nonpolar media, will be used in mechanistic and synthetic studies. These will include further studies of the mechanism of action of flavins in enzyme oxidation systems and the development of an efficient and general macrolide synthesis.

BIBLIOGRAPHIC REFERENCES: W.H. Rastetter and T.J. Richard, 'Generation and Cope Rearrangement of Two Methylated Oxepin Oxides', Tetrahedron Lett., 2995 (1978). W.H. Rastetter and T.J. Richard, 'The Behavior of 2, 7-dimethyl-oxepin Toward Azodicarboxylate Esters: The Fortuitous Synthesis of a Cycloheptatriene from an Oxepin', Tetrahedron Lett., 2999 (1978).

115. TOTAL SYNTHESIS OF THE ANTICANCER AGENT PRETAZETTINE

Martin, S. F., University of Texas, Austin Campus, School of Natural Sciences, Dept. of Chemistry, *200 W. 21st St., Austin, Texas*, 78712, United States of America

Previously established synthetic methodology for the creation of quaternary carbon centers by operations involving the geminal disubstitution at a carbonyl carbon atom is being extended to allow the enantioselective construction of fully substituted carbons. Several of these procedures are also being applied to the syntheses of biologically active natural products, especially alkaloids of the Amaryllidaceae family. Of particular interest is the development of efficient total syntheses of pretazettine which is an anticancer/antiviral agent together with the related alkaloids precriwelline, tazettine, and galanthamine. Selected synthetic intermediates and analogs of pretazettine will be submitted to the National Cancer Institute for biological evaluation.

BIBLIOGRAPHIC REFERENCES: S. F. Martin, T. A. Puckette, J. A. Colapret, J. Org. Chem., 44, 3391 (1979). New Methods for Alkaloid Synthesis. Facile Total Synthesis of (plus or minus)-O-Methyl-joubertiamine and (plus or minus)-Mesembrine.

116. MOLECULAR PHARMACOLOGY OF TUMOR AND VIRUS INHIBITORS

Grollman, A. P., Klett, R., Takeshita, M., Lin, S. Y., State University of New York, Health Science Center at Stony Brook, School of Basic Health Sciences, Dept. of Pharmacological Sciences, *Stony Brook, New York*, 11790, United States of America

The specific objectives of our research are to define the mode of action and structure-activity relationships for certain antitumor and antiviral agents and to explore the molecular basis for their interaction with macromolecules.

The primary agents under investigation at the present time are the bleomycins, a family of structurally related glycopeptide antibiotics which possess antimicrobial, antitumor and antiviral properties.

Determining the mode of action of the bleomycins may be of value in establishing normal pathways of macromolecular synthesis as well as in correlating biochemical properties of drugs with therapeutic activity. Knowledge of the mode of action suggests new uses and may warn against unsuspected toxicities. Information gained from these studies may provide clues that will lead to the development of new chemotherapeutic agents.

117. ANTITUMOR/ANTIBIOTIC SUBSTANCES - SYNTHESIS AND CHEMISTRY

Corey, E. J., Harvard University, Cambridge Campus, School of Arts & Sciences, Dept. of Chemistry, *Cambridge Station, Cambridge, Massachusetts*, 02138, United States of America

There are several objectives of this research. One is to develop chemical syntheses of certain complex molecules showing antitumor, antiviral or antiobiotic activity of an exceptional sort. In order for such syntheses to be practical and sufficiently flexible to allow the production of a wide range of structural analogs, new synthetic reactions and methodology must be developed. A second goal, therefore, is to discover new synthetic principles and methods, in order to extend our capacity to effect syntheses of the most challenging type. In a most general sense we seek to extend the power of organic synthesis upon which much of the progress of medicine ultimately depends and at the same time to achieve the synthesis of important substances.

The proposed research centers around the following biologically active substances or families of substances: 1) maytansines, 2) erythromycins, 3) oleandomycin, 4) aplasmomycin, 5) rifamycins, 6) coriolins, 7) quadrone, 8) ovalicin, 9) interferon inducing substances and 10) cyclic CMP analogs.

Finally, it is our aim, through these chemical studies, to make significant contributions to medicine.

118. STRUCTURE AND ACTION OF THE ANTIVIRAL PROTEIN PAP

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PAP (pokeweed antiviral protein) is known to retard virus replication by enzymatically inhibiting protein synthesis on eukaryotic ribosomes. The effects of its action are very similar to those of the A chain of the anti-tumor enzyme ricin, also under study in our laboratory. The enzymes attack the 60S subunit of the ribosomes but the nature of the catalyzed reaction is totally unknown. In fact it is not even clear whether they recognize ribosomal proteins, RNA or both.

We have crystallized PAP in a form suitable for high resolution x-ray analysis and have obtained three isomorphous heavy atom derivatives of the protein. A 4.5 angstrom unit map has been produced and shows the enzyme to be composed of two roughly cylindrical domains, each of roughly 12 to 15 thousand daltons. We propose to carry this structural analysis to 2.8 angstrom unit resolution using the derivatives presently available and to build a molecular model of the toxin. We hope to compare the structure with that of ricin, for which a 2.8 angstrom unit map is presently available.

BIBLIOGRAPHIC REFERENCES: Irvin, J.D., Kelly, T., and Robertus, J.D., Purification and Properties of a Second Antiviral Protein from Phytolacca americana Which Inactivates Eukaryotic Ribosomes, Archives of Biochemistry and Biophysics, 200 418-425 (1980).

119. STUDIES OF NARCISSUS ALKALOID WITH ANTI-LEUKEMIC ACTIVITY

Furusawa, E., University of Hawaii System, Manoa Campus, John A. Burns School of Medicine, Dept. of Pharmacology, *2444 Dole St., Pacific Biomedical Res. Ctr., Honolulu, Hawaii*, 96822, United States of America

We have found that narcissus alkaloids (pretazettine and pseudolycorine) have a remarkable anti-leukemic activity against Rauscher viral leukemia and a moderate anticancer activity against several transplantable (non-viral) tumors in mice. The purpose of this project is the further basic studies of the alkaloids, especially the mechanisms of therapeutic activity at

enzyme, cellular, and animal levels, evaluation of the therapeutic activity against spontaneous AKR leukemia as a better model of human lymphocytic leukemia, establishment of the best treatment schedule, including the combination with standard anticancer drugs or other investigative agents, prevention of viral expressions and, finally, eradication of the viral genomes from the chronically virus-infected cell lines and animals, pharmacotoxicological studies in bigger animals and synthesis of more active derivatives of the alkaloids by massive production of the alkaloids, and studies of chemical structure-biological activity relationship, etc., to be completed before any future clinical application.

120. HERPES SIMPLEX VIRUS GLYCOPROTEINS AND INFECTION

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The herpes viruses have been firmly associated with several neoplastic as well as cytolytic disease states. In both cases, the glycoproteins of the virus play an important role of allowing the virus to infect a cell and be recognized by host immune defenses. In as such, the research proposed here will center around purification, characterization, and investigation of the function of the individual herpes simplex virus I (HSV) glycoproteins. The individual HSV glycoproteins will be purified by lectin affinity chromatography. The purified glycoproteins will be used for the preparation of monoclonal anti-glycoprotein antibodies. These antibodies and the purified glycoproteins will be used to aid in further purification of the HSV glycoproteins, to study the expression of the specific glycoproteins on HSV infected and transformed cells, to provide a rapid means of clinical quantitation and identification of HSV and will provide a tool to study antibody and T lymphocyte recognition of the HSV transformed or infected cell. In addition, the mechanism of action of a new antiherpetic amantadine derivative will be studied. The mode, mechanism and site of action of inhibition of herpes virus replication will be studied by assaying the effect of the compound on virus production, viral and cellular membrane structure and the lysosomotropic activity of the compound. In addition, establishment of tromantadine resistant mutants will allow functional characterization of another HSV protein.

121. ENZYMOLOGICAL STUDIES OF MAREK'S DISEASE HERPESVIRUS-INFECTED CELLS

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OBJECTIVE: Identify the proteins that are involved in Marek's disease herpesvirus (MDHV) DNA replication and transcription. Initial efforts have resulted in the identification by us of MDHV-induced DNA polymerase in cultured cells infected with the virus. Studies are under way to purify the viral-induced DNA polymerase, to describe its catalytic and structural properties, and to ascertain the role the enzyme plays in viral DNA synthesis. A search for specific inhibitors of MDHV-induced DNA polymerase which might prove to be effective antiviral agents is under way.

APPROACH: A quest for the proteins involved in MDHV DNA synthesis and transcription will begin using cultured cells infected with the virus. These viral-induced proteins will be studied using standard protein chemistry procedures. Once the proteins are identified and characterized, search for the viral-induced proteins in the cells of chicken's with Marek's disease will proceed.

PROGRESS: Phosphonoformate was synthesized in this laboratory and was shown to be an effective inhibitor of the replication of herpesviruses (Marek's disease herpesvirus, the herpesvirus of turkeys, and herpes simplex virus of man). Phosphonoformate inhibits herpesvirus replication by blocking herpesvirus DNA synthesis through a specific inhibition of the herpesvirus-induced DNA polymerase. In animal model systems, phosphonoformate was effective in treating herpesvirus infections in mice, rats and guinea pigs. Tests in a monkey-model system are currently being conducted by the Antiviral Substance Group of the National Institutes of Health.

122. POTENTIAL ANTIVIRAL AGENTS - DYES - PHOTODYNAMIC INACTIVATION OF VIRUSES

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This is part of a broader project. A summary of this subproject is not available.

123. POTENTIAL ANTINEOPLASTIC AGENTS - MAYTANSINE

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This is part of a broader project. A summary of this subproject is not available.

124. POTENTIAL ANTINEOPLASTIC AGENTS - MYXIN

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This is part of a broader project. A summary of this subproject is not available.

125. POTENTIAL ANTIVIRAL AGENTS - BLEOMYCIN

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This is part of a broader project. A summary of this subproject is not available.

126. ANTIVIRAL CHEMOTHERAPEUTIC AGENTS

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This is part of a broader project. A summary of this subproject is not available.

127. NEW ANTIVIRAL AND ANTICANCER AGENTS (MICE)

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This is part of a broader project. A summary of this subproject is not available.

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CURRENT CANCER RESEARCH PROJECT REGISTRATION FORM

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National Cancer Institute
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Bethesda, Maryland 20205

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